

REVIEW OF LITERATURE ON HEALTH HAZARDS OF METALS I. COPPER

BY SARA J. DAVENPORT

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FOREWORD

It is planned to issue from time to time a series of papers on important metals, the mining and processing of which may constitute a health hazard to those exposed thereto. This is the first of the series.

The data on copper in relation to health were collected some years ago in connection with a study of the toxicity of the metal sponsored by the Copper & Brass Research Association. A brief outline of the information was published in 1947 by the Association in a booklet entitled "Copper and Health."

The voluminous data that have been made available for publication by the Bureau of Mines have been revised and brought up to date.

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Sara J. Davenport^{1/}

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INTRODUCTION

The human race has been exposed to copper for thousands of years, as copper and gold were the metals first used by man (1). 1/ According to Ramazzini (2), "of all the metals that man's ingenuity has contrived to dig out of the bowels of the earth, iron and copper are the most useful, far more so than silver and gold." Workings of copper in the oldest known copper mine associated with the smelting of this metal have been traced back to 4300 B. C. Facts revealed by archeological research in India, Austria, Hungary, France, Italy, and Spain indicate that the use of copper probably began about 5000 B.C. (3), and that about 4000 B.C. a copper age came intermediate between the stone and bronze ages (4).

Copper ores are rather widely distributed and are found in nearly every country. Many of the copper mines of Europe have been worked since before the Roman Empire. The most important mines in Europe occur in the district around Huelva, extending into Spain and Portugal. The German deposits are located mainly about Mansfeld (Rhenish Prussia); they have been worked continuously for 1,000 years. Some of the mines in Africa have been worked by the natives for centuries, and China has produced copper from shallow pits for thousands of years. India also has many ancient mines worked in the early centuries (5).

In the New World, long before Columbus discovered America, the original inhabitants toiled in the copper workings of the Andean Cordillera (6). Their best diggings appear to have been at Chiquicamata, Chile, site of a huge present-day open-pit mine.

Philological and archeological research indicates that the primitive Aryans were acquainted with copper, as they apparently had not reached the iron age and there was no Aryan word for tin, an essential constituent of bronze. The Sanscrit avas, the Latin aes, the Gothic aiz, the German Erz, and the English ore are all of Aryan origin. Later the terms were used to denote copper, brass, or bronze but avas, aes, and aiz are thought to be primitive and originally meant copper, although as late as Pliny (75 A.D.) 1 term was used for the 3 substances.

According to the Bible, Tubal-cain, 3870 B.C., was an artificer of brass. After the exodus of the children of Israel from Egypt, while they were wandering in the wilderness of Sinai, Moses called upon them to make an offering of "gold, and silver, and brass" to build the tabernacle. The brass was probably either copper or bronze, as zinc had not been discovered at that time, but the translators of the Bible did not understand the metallurgical distinction of the three terms. It may be of interest to note in this connection that the term "bronze" has been

1/ Underlined figures in parentheses refer to items in the bibliography at the end of this report.

substituted for "brass" in the new Revised Standard Version of the Bible. According to chapter 27 of Exodus, the altar was made of wood overlaid with bronze (Revised Standard Version); and all the vessels of the altar - pans for receiving ashes, shovels, basons, fleshhooks, and firepans - were made of this metal. The bronze that was contributed was 70 talents and 2,400 shekels. With this bronze were made the door of the tent of meeting; the bronze altar and the bronze grating for it; all the utensils of the altar; the bases round the court and the gate of the court; all the pegs of the tabernacle; and all the pegs round about the court. The Israelites probably learned to use copper while in bondage in Egypt, since copper objects have been found in the tomb of one of the earliest Egyptian kings who lived about 4400 B.C. According to Berthelot (7), some of the old Egyptian relics formerly thought to be bronze are made of copper, and in Mesopotamia virtually pure copper was worked about 4000 B.C. Statuettes of copper or copper, with small amounts of tin, have been found dating from about 2600 B.C. Herodotus (8) (who died in 424 B.C.), in writing of his travels in Egypt, mentioned that the Egyptians "drink from cups of bronze and rinse them out every day, and not some only do this but all." However, the earliest authentic bronzes are said (1) to date from about 1600 B.C. In 1933 Bercovitz (2) referred to the use of brass as an ancient custom among Koreans, whose food was served in brass dishes and eaten with brass spoons or chopsticks.

Apparently, about 3000 B.C. the ancient world began to get large supplies of copper from Cyprus, and its copper mines passed successively under the dominion of the Egyptians, Assyrians, Phoenicians, Greeks, Persians, and Romans. The copper obtained from this island was first called aes cyprium, but the term was gradually shortened to cyprium and finally corrupted into cuprum, from which are derived our word copper, the French cuivre, and the German Kupfer. In this manner the primitive Aryan word for copper, aes, has been diverted from its original meaning and preserved in the modern German Erz and the English ore.

POSSIBLE SOURCES OF COPPER POISONING

From the above historical résumé it may be assumed that the human race has had plenty of opportunity to contract poisoning by contact with copper. As soon as men began to mine and work copper they were exposed to any occupational hazard connected therewith. They increased the possible hazard from contact with copper when they began to use the metal to make the vessels in which they cooked their food and the dishes from which they ate it. According to Diodorus (10), who lived about 30 B.C., the Nubian and Ethiopian mines supplied Egypt so plentifully with copper that at the time of the Pharaohs this metal or bronze was used in making domestic furniture, chariots, swords, arrow tips, etc. The present is no exception to the past in the matter of the use of copper, as this has been called the copper age (11). Copper is sprayed on vegetables and shade-trees, used to kill algae in drinking water, is a necessity in electrical work, and is common in plumbing fixtures in the form of brass. Food is canned in copper kettles and kept warm in copper steam tables.

Caddle (12) estimated in 1935 that almost 50,000,000 pounds of copper would be consumed by American manufacturers of mechanical refrigerators. Air conditioning, another new industry, made possible through the use of copper and copper alloys, was predicted to consume several million pounds of these metals with possibility of greatly augmented tonnage in the event of improved business conditions in future years.

Another new use mentioned by Caddle (12) was the electro sheet copper invented about 1935, which was being generally used for weather proofing and dampproofing.

Other uses mentioned were as insulation material and as a protection against termites (copper or copper alloy shields placed between the foundation walls and the superimposed woodwork of buildings will keep termites out). In fact, according to Caddle:

Without copper there would not be possible today most modern inventions which have made this the greatest age in all the history of all the world. Modern civilization would falter, for copper permeates all modern progress. In every day life it is the agent of business and industry and the promoter of our comforts and conveniences.

Most of the earliest statements found regarding the physiological action of copper describe its use as a medicine rather than its effects as a poison. In his *Natural History*, published about 77 A.D., Pliny (13) stated that the healing art profited much from copper mines, for nowhere did ulcers heal more quickly than in their neighborhood; he gave many prescriptions for the use of copper as a medicament but apparently did not discuss its toxicity.

Presumably food has been cooked in copper vessels ever since the metal was discovered and utilized. Galtier (14), in 1855, said that the knowledge of inorganic poisons was not as ancient as that of organic poisons and that it would be very difficult to fix the exact time of the discovery of their toxic effects; however, since Moses recommended that copper vessels be kept absolutely clean he probably knew the symptoms produced by foods prepared in vessels of this metal.

To complicate the question still further, copper was found to be a constant constituent of both plants and animals. In 1893 Tschirch (15) said that -

Our food is prepared in copper vessels, we use copper coins in our daily business, and cover our dining table with copper-containing utensils; indeed even the dust of the room and the laboratory very regularly contains copper, not to mention the numerous medical uses of copper salts. And even further, there is a possibility that all plants contain copper, as it has been found in many. Even our cereals and other important foods and drinks contain not inconsiderable amounts of copper taken from the ground. Undeniably a certain amount of copper is taken into the body daily.

Copper Poisoning in Industry

In a monograph on industrial medicine published in 1567, Paracelsus (16) included copper among the ores that caused various disturbances of the lungs, stomach, and intestines of those engaged in mining and smelting them; these disturbances, he said, were called "miner's diseases."

In 1700 Ramazzini (17) remarked on the green color of the complexion, beard, and hair of the workmen whose business it was to melt and hammer copper and tin.

In 1847 Chevallier (18) reported that an inquiry had been made to find out whether workers who prepared verdigris were subject to particular diseases. Men were found who had worked 20, 30, and 40 to 50 years in the industry without being affected and who declared that none of those who were employed in the industry, including women and young girls, had been attacked by colic or other particular manifestations of disease. None of the doctors of the cities where workers in this industry lived could recall having treated copper colic, although they had frequently cared for attacks of lead and mercury colic. No precautions were taken by the workers. Painters who used the verdigris in colors were of the opinion that painting with verdigris did not result in any symptoms. With reference to other

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copper workers, Chevallier said they were exposed continually to vapors from red hot copper but were never attacked by colic. The coppersmiths who worked with cold copper were continually swallowing considerable quantities of scrapings or copper dust and were subject to copper colic, but this was not nearly as dangerous as colic from lead, as was proved by the robustness of the copper workers in general, who on the average lived as long as other men, and some even became octogenarians. The coppersmiths absorbed so much copper that their bones became greenish or bluish and the hair and urine green. It was concluded that copper, even in a very large dose, was compatible with healthy organs.

Galtier (14) quoted from the newspapers the case of a workman who had contracted copper colic by immersing his hands several times in a solution of copper sulfate to remove zinc plates.

Bochefontaine (19) in 1883 declared that the workers in the copper smelters at Villedieu often had inclination to vomit and suffered from the colic and even diarrhea and that when they gave up their habitual work these symptoms ceased. Because the occupation incriminated by Bochefontaine was one of the principal industries of Paris and because the latter's statements were inexact, Burq (20) undertook to refute the statements in regard to the toxicity of copper. He quoted Thiebault to the effect that apprentices, when they first began work, were attacked by the colic but this was exceptional and in all cases after some months of work the symptoms ceased. According to the records of the society (20), composed entirely of workers in bronze, which was founded in 1819, data taken from the perfectly kept medical register of cases of colic showed that the society had paid since its foundation for 66 days of sickness from colic or other illness, or about 1-1/4 days per year. An annual of the society for the year 1865 gave the following facts: 348 active employees; 170 between 20 and 40 years of age, 115 between 40 and 55, and 63 between 55 and 65. There were 37 pensioners, of whom 31 were between 65 and 75 and 6 between 75 and 90. Similar figures for 1884 were: 271 active members; 111 between 20 and 40; 109 between 40 and 55, and 63 between 55 and 65. There were 54 pensioners of whom 44 were between 65 and 75 and 10 between 75 and 93. Not one of the 54 pensioners had been attacked by any kind of paralysis.

In 1884 Bochefontaine (21) reiterated his belief in copper poisoning. He also quoted the annual report of the Normandy Association as authority for his statements in regard to the effects of copper on the health of workers, that copper emanations were the cause of dyspnea, gastric disturbances, and diarrhea. The green stains on the teeth, the green tint of the hair and chest, the irritation of the gums, the salivation, the thirst, the nausea, and the copper colic observed everywhere among the copper engravers were explained as follows: To finish the chasing, that is, to smooth the surface that the casts presented as they came from the font, the casts were drilled by hand. The drill heated rapidly and did not bite well into the bronze, and the workmen put the instrument into the mouth and wet it with the saliva. The copper dust on the drill points remained on the lips. As this was repeated very often, the quantity of dust remaining in the mouth represented at the end of the day a relatively large amount, so that symptoms of copper poisoning were not long in appearing. Bochefontaine (21) stated that he had mentioned the general paralysis of engravers when alluding to some cases in which there seemed to be no question of the influence of alcoholism but that it was necessary to know that alcoholism was very frequent among these workers.

Lewin (22), in 1900, made the following statement in regard to copper poisoning in industry:

It was in the year 1751 that Desbois gave a mournful description of the injury to health through the absorption of copper by the workers of

Villedieu-les-Poêles. Although it was proved to him immediately that the cases were false or forged it did not help; since that time many other general statements have found place in toxicology, in spite of the objective proof that while the signs of illness described perhaps in many cases are correct in themselves they can be traced back to other causes. It is shown, for example, that in the village of Durfort in the Department of Tarn for many centuries the forging of vessels has been handed down from generation to generation and the men do not become ill thereby and their average duration of life is entirely normal, although everywhere in the place copper dust lies on the streets and from there is spread through the atmosphere. I could give many similar reports.

The morbidity of the Berlin coppersmiths (from 1887 to 1897 the local sickness insurance society had 4,780 members) was less than that of the average Berlin industrial worker. Among the latter 24.4 percent suffered from respiratory diseases while among the coppersmiths only 16.71 percent were affected.

Lewin stated that, in spite of all such positive proof of the harmlessness of copper dust, many assertions were made from the middle of the 18th century until recent years in regard to the harmfulness of copper. The known symptoms of lead poisoning were transferred to copper, and a pathology of chronic copper poisoning was constructed that differed from lead poisoning only in name.

Lewin himself made observations among representatives of all branches of work with crude copper and brass, from the casting to the drawing of tubes, rolling, scraping, pickling, trimming, turning, annealing, forging, and polishing. In these industries he found not a few who had been engaged in the same work for 5 to 40 years. Also, during the 10 years before this report was made, he visited small bronze foundries in which the hygienic conditions were very much worse than in the industries mentioned, and for a long time he chiseled or filed bronze castings and did other work of a coppersmith in connection with which he breathed in a small amount of copper dust. Among the many copper and brass workers he had seen, questioned, and examined, who had had absolutely no motive to conceal any suffering they might have incurred in the industry, Lewin found none who suffered from chronic copper poisoning or who exhibited any kind of general disturbance having the stamp of disease that appeared either permanently or temporarily. He found that, where it was reported that there was chronic copper poisoning, some other material than copper was concerned or with certain operations some individuals had an acute quickly disappearing illness but not a chronic one.

The following statement was made by Ramazzini (2):

So many and so inexplicable are the mixtures of metals confined in the bowels of the earth that it is impossible to determine precisely what injurious element is present in this or that mine and how it is that one part rather than another is affected.

In regard to acute poisoning from copper dust, Lewin (22) stated that the amounts of copper dust stirred up from the floor would not cause acute symptoms in man as the greater part stirred up by sweeping, on account of weight, does not reach the nose or mouth and quickly settles to the floor again. However, if a dog runs around in such a room he is affected by the metal dust. This is indicated by the fact that he attempts to come out again and because he vomits after coming out if he has remained in such a room for awhile. Workers may suffer in a similar way under special conditions in which they breathe metallic copper and suboxide of copper. This, Lewin said, is possible in manufacturing copper vessels and especially so with

the sifting of copper sweepings, in the latter case even when a damp sponge is worn over mouth and nose.

In 1914 Harnack described a case of chronic copper poisoning from a bad gold filling in the cavity of a tooth. A woman had gone to a dentist in 1908 and had a bridge made, supposedly of as pure gold as possible. After wearing the bridge for 3 months she began to suffer apparently from a bad root, with inflammation of the middle ear. This formerly healthy young woman gradually became ill, and by 1912 the illness had reached a severe stage. There appeared especially a severe affection of the stomach and intestines with vomiting, complete loss of appetite, severe colic with painful tenesmus, and considerable bloating; then muscular tremor, great weakness of the extremities, and general loss of strength; and finally difficulty in breathing, irritating cough, and tendency to catch cold. When the bridge finally was removed the root canal after 3 weeks still had a penetrating verdigris odor. At the time of the report the bridge had not been worn since January 1913, and the health of the patient since that time had gradually improved. A green color at the place where the bridge touched the gums and the typical odor led to the belief that the alloy must contain very little gold; test showed that it was scarcely 8 carats, two-thirds being of common metals, principally copper. Harnack said that he did not see the woman during the course of the disease but had this information from her afterward by oral and written communication.

In referring to the above case in 1933, Haase and Ulsamer (24) said that the illness ascribed to the copper might have been due to an entirely different cause, as it is well known that carious teeth are the portal of entrance into the body for all sorts of poisons.

According to Thompson (25), copper miners and smelters sometimes acquire intestinal cramps, diarrhea, vertigo, and an uncertain gait. A green-blue line forms at the margins of the gums, and the hair may be colored green. However, he concluded by saying that many of the symptoms attributed to copper probably were due to associated metals.

In 1917 Preble (26) found that copper fumes, when habitually inhaled in the industries, had never been known to cause a chronic metallic intoxication, although it stained the gums green and caused a metallic taste.

In 1929 the editor of the Journal of the American Medical Association (27) was asked what percentage of girls working in telephone offices as telephone operators and using the brass telephone plugs in the usual way had dermatitis from contact with the brass plugs. Also, the editor was asked whether men working with brass in pump factories knocking the rough parts from castings were liable to have dermatitis or other skin trouble resulting from such contact with brass. The answer was that, although the notion was widely prevalent that brass and copper are responsible for a dermatitis and that infection is promoted by contact with instruments made of this metal, for example, picking a splinter with a brass pin, it was highly probably that a dermatitis seldom arose from contact with brass materials unless they were dusty or oily; in the case of telephone operators, dermatitis due to handling brass plugs was unknown. However, men working in brass foundries engaged in knocking the rough parts from castings were liable to incur a dermatitis from contact with dusts. This may occur as a result of mechanical action when the dust of any metal is present but appears to be more frequent in copper or brass works. A dermatitis due to zinc oxide has been described, and it is possible that there may be considerable zinc dust in the dusts of rough brass castings.

Kröner (28) said that copper poisoning by inhalation had not been observed with certainty up to that time (1931).

In 1932 Eisner (29) reported a case of dermatitis of the face (especially the forehead), hands, and forearms of a man exposed to dust containing 49.3 percent copper. Flap tests on the intact skin gave a positive reaction from the application of 10 to 25 percent copper sulfate solution. The dermatitis was healed by desensitization treatment with copper sulfate solutions.

In 1932 Gudjonsson (30) described four cases of poisoning among workmen engaged in polishing brass ship propellers. The work was carried on in a small room "under primitive conditions." There was much metal dust in the room, which was ventilated through a fan in the window. Apparently polishing metal ship propellers has a bad effect on the health of the worker; the metal dust in the room seemingly caused the disagreeable symptoms, and the question rose as to whether the patients were suffering from chronic copper poisoning.

Gudjonsson (30) stated that metallic copper is not now considered as especially poisonous and quoted Hofmann and Lehmann to the effect that an adult should be able to take 100 to 120 mg. of copper daily without becoming ill, although according to Zangger copper poisoning can occur through breathing dust containing copper, and it is then similar to poisoning by mouth. The four cases of poisoning among workmen engaged in polishing brass ship propellers were described by Gudjonsson as follows:

Case 1 was 19 years old and in 1929 had worked for 2 months polishing ship propellers. The first week he observed nothing but after that became extraordinarily sleepy and later slept many times while at work. At the same time he suffered from headache, vomiting, dizziness, and pains in the stomach. His face and hands were green from metal dust and he always had furuncles on the face around the mask he wore during work. He came to the hospital where he was operated on for appendicitis. Afterward he obtained other work and has remained well since. As he previously was always well he thinks that the above-mentioned disagreeable symptoms were due to the bad conditions of the working place. Nothing was found on microscopic examination of the appendix, which indicated that the metal dust was the cause of the pain.

Case 2 was 19 years old. In 1931 he had worked 2 months at polishing. After about 14 days he began to be dizzy and to have pains in the head; once on the street on the way home from work he fainted. The dust bothered him very much, in spite of the fact that he wore a mask, and he always had a sweet metallic taste in his mouth. The skin of his face was always very green and there were furuncles where the edge of the mask touched his face. He thought, as did the first case, that this distress was caused by the work, since he was afflicted similarly neither before nor afterward.

Case 3 was 17 years old. Polished ship propellers for 2 months. The dust was very disagreeable to him. The first month he noticed nothing. Then began pains in the stomach, dizziness, poor appetite, headache, and severe sweating. The skin of his face was green around the mask. After leaving the work he recovered completely and has never had similar symptoms since. Thinks that the work was the cause.

Case 4 was 21 years old. Worked in 1930 for 3 months polishing ship propellers. The first three weeks he observed nothing, but took cold and had suffered afterward from continual coughing, noted dust in the throat, sweet metallic taste in the mouth, and got a red band on the skin around the mask. The symptoms disappeared after he left the

work. Like the others, he was certain that the conditions of the working place caused the distressing symptoms.

Gudjonsson believed that irritation from the action of the sharp metal dust was due to small superficial lesions and maceration caused by corroding or chemical action and not the physical action of the dust as other mechanical action of the dust in these cases was not very likely. He excluded any chemical action of the dust of the polishing medium. On the other hand, he thought it possible that the symptoms might have been due to poisoning by metal dust, and as the material contained about 90 percent copper and 10 percent tin it was a question of copper poisoning. He had the following to say in this regard:

Opinion from practical experience is rather in disagreement as to whether copper can cause poisoning in the amounts which workers usually breathe or to which they are exposed. In some cases it appears possible. In the cases given here there was no sign of the typical copper poisoning with its acute gastro-intestinal course. Two of the workers, however, had pains in the stomach, in one case accompanied by vomitings. In the latter case the symptoms perhaps were due to chronic appendicitis. Therefore the cases described were not due to acute copper poisoning. Up to this time chronic copper poisoning has been considered as extremely rare, if it has ever been observed with certainty. Whether it is the case here one dare not say. It is interesting that three of the four workers had symptoms of the central nervous system, dizziness, and headache and one suffered from sleepyness. All the workers were young and strong men, so that these symptoms could scarcely have been accidental.

Gudjonsson thought it would be interesting to learn whether experiences in other places point in the same direction as those reported here.

Filehne (31) experimented to determine the toxicity of copper dust that might be breathed by coppersmiths or other workers in copper. The conclusion reached from these experiments (made on dogs) was that breathing fine copper powder is hazardous, and it is advisable to protect the workers. To prevent poisoning he recommended that the worker should gargle and clean his hands thoroughly before eating.

According to Mallory (32) the sources of poisoning are distilled liquors contaminated with copper dissolved from the copper worm of the condenser by the action of volatile organic acids (citric, acetic); occupations involving exposure to copper dust (brass foundries, brass milling, planing and polishing, telephone lines-repairing) since the metal is readily dissolved in the liquids of the body however it may obtain entrance; and acid foods, jellies, and candies contaminated by being cooked in copper vessels.

Kröner (28) stated that up to 1931 cases of copper poisoning described have almost always been due to copper salts reabsorbed by the gastrointestinal tract.

Kröner stated that:

People have grown accustomed to copper through long use of utensils made of this metal. Hofmann, in Textbook of Inorganic Chemistry published in 1918, says that an adult can stand daily without harm 100 mg. copper; the amount of copper added to green preserved vegetables is below this limit. Larger amounts of copper salts cause vomiting, gastric pains, and bloody diarrhea. According to statements of the

daily press, these symptoms appear more frequently in localities in which plum jam is made, usually in copper vessels, when the product is left too long in the vessels so that considerable quantities of copper compounds can be formed with organic acids under the action of the oxygen of the air.

The real effects of the heavy metals are not so marked with copper as, for example, with lead or mercury. Copper poisoning by inhalation has not been observed with certainty up to this time. According to Zangger, in Textbook on Toxicology published in 1928, workers certainly must have breathed dust containing copper and experienced the symptoms mentioned above. However, other metals are not excluded from being present. Copper spray, which is used in large measure to prevent damage in fruit and grape culture, has caused no noticeable injury. However, the conditions for bringing about poisoning by inhalation of the sprayed liquid are very unfavorable, since the worker remains in the open air and the dust solution is caught by the plants.

I have had the opportunity to observe an entire series of cases of slight poisoning which undoubtedly were caused by breathing a very fine spray containing copper. A solution of copper hydroxide in ammonia, used for a special purpose in an industrial laboratory, was sprayed with a compressed air gun at a pressure of about 3 atmospheres. The solution contained about 0.06 to 0.1 percent copper and 0.1 to 0.2 percent ammonia. The distribution of the liquid was so fine that only a very small amount of it could be recovered with the use of a thick tin plate. The capacity of the gun was probably 1 liter per hour and the workroom was about 10 m. long and wide and 6 m. high. In a quarter of an hour after beginning to use the gun characteristic difficulty in breathing, pain in the chest, and metallic taste were experienced by those employed in the laboratory. All persons present were affected in the same degree. With a short stay in the open air the symptoms disappeared. It is very unlikely that the ammonia had a part in causing the symptoms. The concentration of ammonia was too small. I was able to convince myself and the employees that moderately high concentrations of ammonia in the air breathed can be borne without injury.

Schlotz (33) reported in 1948 that workers in a paint and varnish factory in Oslo suffered from what he called "copper fume fever," resembling "brass founder's ague." A feverish condition occurred after pulverization of pure cuprous oxide, which was used in the production of "bottom paint" to protect the external hulls of ships. During the last years of the war it was difficult, often impossible at times, to get head masks. Altogether seven workers suffered from the attacks, which came constantly after every pulverization. Later the attacks occurred rarely. Schlotz made the following statement in regard to the occurrence of copper fume fever:

Copper fume fever has been described in copper founders from France, United States of America, and Russia. Copper fever has also been seen in workmen engaged in the hot rolling of copper in the United States of America and Germany and in spraying copper compounds in the last named country. Metal fever produced by dry, pure metallic dust is known as zinc fever and has been reported from Germany, United States of America, and Norway; as iron fever in Norway and copper fever in Sweden and Norway. It seems more correct

to give the condition the comprehensive designation "metal fever" instead of "metal fume fever," the name used hitherto in medical terminology.

Wallgren and Gorbатов (34) studied 20 patients with symptoms indicating metal fume fever. Seventeen of them were employed in the copper industry, and three worked with zinc or brass. Typical metal fume fever was observed in 13, whereas 7 had only mild or indistinct symptoms. The mean figure of 126 $\pm$ 11 micrograms per 100 cc. is taken as the basis for the evaluation of the serum copper values obtained. In 8 of the 13 cases of metal fume fever, serum copper exceeding 160 micrograms per 100 cc. was noted--this is definitely pathological. In 3 of the remaining 5 cases, in which the serum values were normal, the symptoms were caused by zinc. The increased serum copper values are assumed to be the direct result of inhalation and absorption of copper dust. Particles of metallic and oxidized copper are present in the dust of factories. The serum copper determinations were performed according to the carbonate.

In 1933 Gordon and Rabinowitch (35) stated that, in spite of conflicting data, there is reason to believe that excess quantities of copper may be harmful and that it is rather difficult to reconcile with the "brassy" taste, the green line on the gums, and the green discoloration of the sweat, frequently found among workers in copper mines, the views of industrial-poisoning investigators in the United States that the ill health found among such workers is largely due to other environmental conditions; nor are the views held by the American authorities generally accepted. They report a case of yellow atrophy of the liver in a laborer who had complained for the past 15 years of some gastrointestinal condition. The jaundice, which dated from about 5 weeks before admission, gradually increased in intensity; there was a history of occasional slight attacks during the past 15 years. The history was otherwise irrelevant, except that the patient had worked in a copper mine about 15 years previously, and his illness apparently dated from that period. The amount of copper present in the liver was the largest amount on record as far as was then known.

Copper Poisoning Through Food and Water

in Contact With the Metal

Copper Cooking Vessels and Addition of Copper to Food

There seem to have been much uncertainty and controversy regarding the danger of using copper vessels for cooking and preserving. Galippe (36) in 1877 considered that the results he obtained by feeding dogs copper salts showed the foolishness of the pretended danger of using copper vessels for the preparation of food and that it was not the copper but the zinc plating that was impure and contained arsenic. His conclusion was that copper is not toxic and that, moreover, the disagreeable taste of copper salts when dissolved would prevent their absorption by immediate warning.

In 1883, in a discussion of the use of copper in canned goods, exception was taken to the results reported by Galippe. Brouardel (37) said that he believed that Galippe had said that when there was too much copper in the conserves they were not edible. Galippe replied that he did not remember having said this. Brouardel then said that it had been assumed that taste was enough to determine whether or not there was too much copper in such foods and therefore tolerance would be limited by the taste of the consumer; however, there were canned fresh peas containing up to 15 and 20 cg. of copper per kilogram that were perfectly edible. Galippe replied that he did not believe that even in this case the conserves would be toxic. He based his opinion on the report of Gallard who had eaten "greened" conserves, which

contained a considerable quantity of copper, without finding the taste disagreeable or without being otherwise inconvenienced.

Although Bochefontaine (19) claimed in 1883 that the workers in the copper smelters were affected by the copper, he did not mention any ill effects to the inhabitants of Villedieu from the spoons, forks, plates, frying pans, kettles, faucets, and other copper objects that they used daily. In 1884, however, Bochefontaine (38) reported to the medical society an instance of the mother of a doctor who tasted some preserves sticking to the bottom of a copper kettle and died shortly thereafter with symptoms of acute poisoning, which the doctors attributed to the copper. However, Bochefontaine said there was no autopsy or chemical analysis and that he remembered that the cooking of currant preserves in copper vessels was a very common custom in the Manche Department as well as elsewhere and so far as he knew a case of poisoning by preserves had never been noted.

Galippe (39) was particularly struck by the instance presented by Bochefontaine of the case of poisoning by preserves cooked in a copper vessel. He said there was no proof that this was true, as was admitted by Bochefontaine himself. According to Galippe, everybody knew that it was the custom to cook preserves in copper vessels and that it was the privilege of the children to clean out the residue adhering to the bottom of the vessel, and there was perhaps no one who had not permitted this without causing the least symptom.

Filehne (31) quoted Lehmann who with other experimental subjects consumed 0.02 to 0.03 gm. of Cu daily in coppered preserves without any effect. Once when he ate some peas to which he had added copper himself, vomiting resulted, but there were no further aftereffects.

Tschirch (15) went into considerable detail in regard to the greening of canned vegetables and fruits by using copper, a custom said to have originated in France. According to Tschirch, the French placed on the market the first preserved food colored a vivid green, and the question of copper had been treated by hygienists there for 20 years before Tschirch's publication. They even had a special designation for the coppering of preserves, calling it "verdissage" (greening) or "reverdissage" (regreening). Tschirch considered that only the coppering of such canned goods as peas, beans, cucumbers, etc., was important and that this was practiced to great extent not only in France, but in Germany, Austria, Switzerland, Italy, and England. In reply to the question as to whether this coppering should be allowed or forbidden, he quoted investigators like Gautier and Bergeron, who, although they were not as much disturbed about it as some others, thought it should not be allowed without further investigation. Bergeron protested against drawing the conclusion that regreening was harmless, because it had been observed that small amounts of copper salts in the animal organism caused no toxic symptoms. Gautier thought that the introduction of small quantities of copper in canned vegetables for regreening should be prohibited from the point of view of a rigorous prudence and the rules of strict hygiene as their harmlessness had not been demonstrated absolutely. Also Kobert, although serious poisoning from canned goods containing copper was scarcely to be feared, thought that the pharmacologists should put up an energetic front against the hygienists who permitted the use of copper and demand laws completely forbidding such use of copper. However, Tschirch was of the opinion that, since copper was added to the body from food without the addition of copper to the food, a plausible case of poisoning had never been described in the literature, in spite of the millions of cans of coppered preserves eaten daily, and it would be impossible to eliminate the copper vessel from the kitchen and the factory, it would be better to determine the maximum content that was permissible. He insisted that however they might pretend, copper could not be

eliminated from food. In fact, he said that to entirely forbid copper in food and drink would mean that plants would have to be forbidden to absorb it from the soil and bread and chocolate would have to be labeled as unhealthy. Tschirch first made clear that the addition of copper sulfate to green vegetables preserved their color by forming a compound with the chlorophyll, termed by him phyllocyanate of copper, and that the amount of salt that had to be added to be effective varied with the depth of the color of the fresh vegetables. Drummond (40) quoted Tunicliffe to the effect that on an average 50 mg. per kilogram was enough (that is, expressed in terms of Cu), which was of the same order as the 55 mg. per kilogram suggested as the highest limit to be added to canned goods set by the German official inquiry of 1910.

According to Preble (26), all alleged cases of acute or chronic poisoning from using copper cooking utensils or consuming canned vegetables colored by copper salts were at that time (1917) regarded as due to other causes, such as ptomaines or the lead in the solder of the cans. He also said that in certain countries the use of copper salts to "green" vegetables was legalized.

In Bucharest in 1924 (41) a grocer was prosecuted in police court and convicted and fined for selling peas and preserved pickles adulterated with copper sulfate to the extent of 0.2 gm. to a pound of peas and 0.16 gm. to a pound of pickles. The Rumanian food hygiene law of 1899 recommended that in no case should more than 0.03 gm. of metallic copper be permissible in 1 pound of canned vegetables. The prosecuting health officer admitted that he knew of no case in which injury had resulted from coppered pickles. He stated that the ordinary dose of copper sulfate as an astringent was 0.03 to 0.1 gm., therefore anyone eating 1 pound of the peas or pickles in question would swallow double the medicinal dose. He suggested that copper should be used with extreme caution as a medicinal agent, as of late years other remedial methods and nontoxic astringent drugs had been introduced into practice.

Mills (42), as director of the research department of a medical college in Korea, became interested in the relation of copper to disease and realized that the Korean people, numbering about 17,000,000, were voluntarily performing an experiment along this line in their everyday life by their habits of preparing and eating food. Brass was the universal material from which they manufactured rice and other food bowls, spoons, and some of the kitchen utensils. Wine bottles, some of the stills for making fermented liquors, and a form of chafing dish also were made of this substance. Remnants of food were frequently left in the bowls from one meal to the next; the nature of some of the foods was such that they would probably exert a solvent action on the brass. Children were given more or less solid food within a few days after birth, and the constant ingestion of copper continued until death. The life span seemed to be reasonably long, about 2 dozen centenarians having been listed by the authorities a few years ago. The people believed that the use of brass utensils was in some way a protection against disease; a family might be so reduced financially that it was compelled to sell off most of its possessions, but the brass dishes were the last to go.

King and Etzel (43) in 1927 investigated the copper content of three types of foods commonly prepared in copper or brass equipment: (1) 4 groups of acid fruit products representing different trade practices, (2) milk from 9 different plants, and (3) carbonated beverages from 2 plants. In one of the plants, which he designates as plant 3, the following quantities of copper were found: Plum jelly, 55 p.p.m.; strawberry jelly, 60 p.p.m.; raspberry jelly, 80 p.p.m.; raspberry preserves, 45 p.p.m.; apple butter, 110 p.p.m. It is stated that eating 100 gm. per day of such products as these would alone cause one to exceed the limit of copper

(5 to 10 mg. per day) estimated by Mallory to produce toxic results, without taking into consideration what would be normally supplied in natural foods.

Tests on carbonated beverages showed that normally very little copper is dissolved.

Copper Water-Supply Systems

Another possible source of ingestion of copper is by use in water conducted through copper piping. In 1925 Thresh's (44) approval was asked on the installation of a copper water-plant in a large institution that was similar to the plant that had been in use for many years in a small institution. After communicating with people using copper pipes for water and inspecting the installation used in the small institution, he expressed the opinion that copper pipes could be used with perfect safety. Meantime, a careful search was made for records of the action of copper on the human body, and it was only in out-of-date publications that the opinion was expressed that cases of chronic copper poisoning occurred from the ingestion of minute quantities of copper; in these cases the poisoning never appeared to arise from the use of copper pipes for water or copper vessels for cooking, although until comparatively recently there seemed to have been a widely held opinion that copper was as poisonous as lead; however, this opinion began to be doubted when the causes of food poisoning were investigated. Before this period, if any article of food or drink had caused illness and had been in contact with copper, no further investigation was deemed necessary; the poisoning was attributed to the copper. Dixon's Manual of Pharmacology was quoted as saying that "there is no evidence that man is ever affected with chronic copper poisoning, so that the use of copper cooking vessels and the employment of minute amounts of copper to improve the appearance of peas may not be so harmful as might be supposed." Mamm's Forensic Medicine and Toxicology was quoted also as saying that modern authorities, with few exceptions, state that chronic copper poisoning is unknown. Lehmann calculated that each individual takes into his body 20 mg. of copper, and Tschirch, confirming this, added that, as long as the total quantity of copper ingested did not exceed 100 mg. daily, there could be no question of injury to health. Lehmann, in Methods of Practical Hygiene, according to Thresh, said that copper was virtually harmless and that poisoning in the chronic form probably never existed. Thresh's conclusions on the use of copper pipes for hot and cold water were that copper is acted upon far less by water than is iron or lead; that, being vastly less deleterious than lead, copper pipes are far safer than lead pipes; that copper pipes can be used for all waters that are not acid in reaction; that acid waters should never be used until the acidity has been removed by treatment, and having been so treated copper service pipes can be used; that virtually all waters take up traces of copper if allowed to stand long enough in contact with it, but that under all ordinary circumstances the amount taken up is far too small to endanger health.

An editorial in the Boston Medical and Surgical Journal (45) stated that, although careful analyses of drinking water show copper to be present in minute amounts and the slow corrosion of brass pipes, so common in modern homes, is an added source of the metal, the danger from water and water pipes is slight. Copper is readily attacked by the various acids present in foods, such as acetic, citric, malic, tartaric, and oleic; consequently, any food or drink containing acid will dissolve a certain amount of copper if it comes in contact with that metal. The editorial stated that there is no doubt that the human organism can take care of minute amounts of copper, but where the dividing line is between the harmless and the harmful amount cannot yet be said, and individual susceptibility probably enters in to complicate the problem. However, from what is known of the solubility of copper and the amount of it present in various substances, it is fairly safe to say that the danger from copper and brass pipes is negligible, that copper cooking

utensils might possibly be dangerous, especially when used for fruits, but that the real risk comes from industrial hazards and from moonshine liquor.

The conversion of metal from the metallic to the nonmetallic state is not limited to the solvent action of acids or bases but proceeds in salt solutions of the most varied concentration and even in virtually salt-free distilled water, according to Bleyer and Schwaibold (46). The ever-increasing utilization of metallic raw materials for preparing and preserving foodstuffs directs attention to the corrosion process, on the one hand to find the most suitable and durable metal and on the other to find what amounts of the technically satisfactory metals are changed by the food and drink to the nonmetallic state and in this event the possible effects physiologically valuable or injurious and what changes in taste and color can be expected. In the investigation by Bleyer and Schwaibold, it was deemed important to determine the beginning of corrosion in metal vessels where the liquids mostly act on the metals for a short time. The intensity of the beginning reaction with drinking water at 18° and 75° and with tea and coffee at 75° is presented by these investigations in tabular form.

A tin container coated on the inside with copper is the most effective of all types of containers investigated by Nitardy and Billheimer (47) for preserving anesthetic ether in its original state of purity. Ether, originally containing a small amount of ether peroxide, when stored in any sealed container will eventually become free from peroxides and simultaneously traces of acids develop, probably acetic acid, due to decomposition of peroxides. If stored in a copper container, this change is accelerated. Ether, originally free from peroxides, when stored in contact with copper is not subject to oxidation and is maintained in its original state of purity for an indefinite period. Any oxygen that may be dissolved in the ether or present in the can disappears during storage, being removed by combination with the copper.

In a study of copper from the physical, chemical, and health standpoints when used in the construction of water-supply systems, Haase and Ulsamer (24) list the following places in this connection in which copper is used: For conveying water to the filter plant for treatment, for suction pipes, and for filtering material; for conducting the filtered water, for boilers and heating coils for heating systems, for condensers and cooling coils for cooling systems, for pans and pots for cooking. The uses of copper are very numerous in the water-supply field, as, for example, for armatures, fittings, watermeters, stopcocks, floating valves, and flushing vats, all of which have proved satisfactory. The findings from the chemical point of view on the use of copper as a material for construction of water-supply systems are summarized as follows:

1. In all waters an oxide protective layer consisting mainly of cuprous oxide is formed on copper.
2. The hardness of the water and its content in natural salts and gases determine the time required for the formation of this protective oxide.
 - a. In very acid waters and in waters with a strong gas content, it may be several years before the protective formation occurs, for which reason copper is not recommended as a piping material for such waters. However, such waters are not used by central supply systems without preliminary treatment by which they acquire the properties of normal waters.

b. In normal soft surface and ground waters the oxide layer forms within a year at the latest if enough oxygen is present, hence copper can be recommended for such waters.

c. In moderately hard and hard waters the layer forms within even a few weeks, and there is no objection to the use of copper.

3. Until complete formation of the protective layer, the copper content is regulated by the chemical composition of the water; the harder the water the less the copper content.

Distinction must be made between actually dissolved copper compounds that can be tasted and tasteless suspended compounds of copper. The latter are readily formed in waters of more than 4° hardness.

4. After the formation of the protective layer, the copper content in warm and cold flowing water as a rule amounts to not more than 0.3 or less than 0.1 mg. per liter.

5. Incrustations or deposits in excess of the usual protective layer do not occur with copper in either warm or cold water; the opposite is true of iron which is chemically less resistant.

Copper Apparatus for Preparation of Dairy Products

The solubility of metals in milk and other dairy products is interesting, both from the standpoint of the durability of the equipment and of the effect of dissolved metals on the flavor, keeping quality, and healthfulness of the products. Formerly the problem was considered primarily of interest to the manufacturer of dairy equipment; but, with the keener appreciation of the possible effects of the dissolved metals on the dairy products in recent years, the problem became of direct interest to the manufacturer of dairy products.

In 1905 Golding and Feilmann (48) reported that milk from churns returned from London to Leicestershire with the complaint that a flavor had developed and was driving away the custom of the retailer was found to have been affected by an old cooler, much of the tin of which had worn off, exposing the copper. The action of milk on copper was investigated to determine whether the flavor was due to the copper only or to the combined action of copper and other factors. It was found that milk when passed over the cooler took up small quantities of copper (in one case 2.5 p.p.m.) and, although no flavor could be detected immediately, the characteristic flavor invariably developed after the milk had stood for about 18 hours at room temperature. Fresh milk (600 cc.) left standing over a piece of clean copper foil (15 by 11 cm.) for 1 hour contained 3.5 to 4 p.p.m. of copper, while copper foil left in milk for 36 hours in an open vessel resulted in a solution of 45 parts of copper per million of milk. It was also found that air greatly aided in the solution of the copper in milk. Clean copper gauze, fixed on the surface of some milk in a beaker and left for 1 week, resulted in the solution of 121 p.p.m. of copper, while in a similar experiment in which the copper gauze was kept at the bottom of the beaker only 48 p.p.m. of copper were found in milk in contact with 10 gm. of clean copper turnings. Leaving the milk in contact with the copper for 1 week resulted in the solution of 45 p.p.m. of copper; in a duplicate experiment, but without air being passed through, 10 p.p.m. only were dissolved. In all these cases a flavor similar to that under investigation developed in 12 to 18 hours and

became more pronounced in the course of a few days. Good keeping properties were also imparted to the milk. Fresh milk straight from the cow was taken and clean glass vessels, not dairy utensils, were used. When, however, the milk was run into a vessel containing many lactic ferments no flavor was developed, and the milk soured. Separated milk containing 0.25 percent of lactic acid dissolved in 44 hours 78 p.p.m. of copper with air and 49 p.p.m. without air. Copper oxide also dissolves in milk in the cold, and the characteristic flavor can develop in such milk. The same is true when small quantities of copper sulfate are dissolved in milk. The blue color produced in milk when much copper is in solution from any of the above sources gradually disappears, according to the authors, on standing in the open or on warming, the characteristic flavor becoming very marked. This flavor seems to be due in part to the development of microorganisms in the presence of copper, which both checks the development of the lactic organisms and plays a more direct part in the actual development of the flavor.

Rice and Miscall (49) gave the following summary of some of the literature on the effect of copper on dairy products:

There are many flavor defects in milk products that have been attributed by investigators to the presence of copper or other metals. Seldom is the flavor directly from the metal itself but through what has been supposed to be a catalytic effect some of the milk constituents are broken down and compounds with disagreeable odor and taste are produced. In 1905 Golding and Feilman described an "alkaline mealy flavor" which they found in certain samples of market milk. The trouble was traced to the use of a cooler from which the tin was worn thus exposing a copper surface; the presence of small amounts of copper dissolved in the milk together with the activity of certain organisms was believed to be the cause of the development of the peculiar flavor. The fact that the flavor was noted only after about 16 or 18 hours gave proof that it was not due to the copper salt itself, but that the copper in some way caused or assisted in a change which resulted in the formation of a substance which rendered the flavor objectionable.

Rogers, Berg, Potteliger, and Davis found that the scores on the butter made from cream to which copper had been added, even in the small amount of 1 mg. of copper per kilo of cream, were poorer than the scores on the control butters.

Hunziker and Hosman by allowing butter to stand in contact with certain metals and their hydroxides found tallowiness developing invariably when copper was the metal. Palmer and Combs added copper lactate to cream in small amounts and proved that butter made from this cream became tallowy and similar results were also obtained by Rosengren. Olson found that the action of rennet on milk was slowed down by the presence of copper and Gerber reported that among other salts those of copper affected the rate of coagulation of milk by various vegetable and animal ferments. Hess, and Hess and Unger discovered the interesting fact that where milk has become contaminated with copper the activity of the antiscorbutic vitamin is reduced.

Changes brought about by contamination of copper and other metals on foods other than dairy products have been demonstrated by various investigators particularly Emery and Henley. They have pointed out what seems to be generally true, that the metals

themselves do not bring about the decompositions except in presence of air or oxygen.

The purpose of the investigation by Rice and Miscall was to consider whole milk merely as a solvent and to determine the amount of dissolved copper in the fluid when in contact with the metal under various conditions. They summarize the results as follows:

The presence of air and oxygen was found to increase enormously the amount of copper dissolved by milk. * * * * * Copper corroded with an oxide surface yielded much more of the metal to the milk than smooth bright copper. It is particularly important to keep such equipment clean and well polished. Since the chlorine disinfectants such as chlorinated soda are usually active in corroding copper surfaces, their use should be avoided on copper hot wells and vacuum pans. Carbon dioxide does not influence the dissolving power of the milk. The amount of copper taken up by the milk increases with the amount of copper surface exposed, and with the time of exposure.

About the same amount of copper is dissolved in the milk at room temperature as at boiling; at 145° F. considerably more copper is dissolved than at either. Therefore, in the manufacture of condensed and evaporated milk where copper hot wells are employed it is important to bring the temperature of the milk to boiling as rapidly as possible. Inasmuch as milk once boiled dissolves about the same amount of copper at 145° F. as at boiling temperature, some transformation or loss of the agency which attacks the copper is indicated. At the present no conclusions can be drawn as to the cause of this phenomenon. It has only been shown that replacing the air in boiled and cooled milk does not reestablish the same solvent power exhibited at 145° F. in unboiled milk.

Milk slightly sour dissolves but little more copper than normal milk. On passing milk containing copper through a separator, the metal distributes itself between the cream and skim milk fractions approximately in proportion to the water. It would seem, therefore, that it is dissolved in the water. The results show conclusively that the copper does not go with the fat, and is, therefore, not dissolved in the fat. Analyses of commercial samples of condensed and evaporated milk indicate that copper is taken up in considerable quantities in the manufacture of these products. From theoretical considerations and from experimental results it was shown that copper may be lost from milk to tin when it comes in contact with this metal, and also that less copper is dissolved when the milk is exposed to surfaces of copper and tin together. Consequently, it would be expected that condensed and evaporated milk could lose some of its copper to the tin of the container and consequently a can purchased on the market would show somewhat less copper in solution than was present at the time of manufacture. Also a copper cooler or pasteurizer imperfectly tinned would yield less copper to solution than if the milk were exposed to the same surface of copper without the tin. On the basis of the same theoretical considerations the tin should dissolve in the milk much more rapidly when in contact with the copper, which would in part account for the rapid wearing of tin from such equipment. The latter point, however, is not supported

by any experimental evidence in this paper, no determinations for tin having been made in any samples.

To determine the influence of temperature on the rate of corrosion of copper by milk under plant conditions, Quam, Soloman, and Hellwig (50) established the time factor at 30 minutes and took the average of a large number of determinations as the measure of corrosion. The solution of copper in milk increased with rise in temperature to a maximum of 85° to 90° C. The break in the curve probably was due to the decreasing solubility of oxygen as shown by Speller, for the corrosion of iron in natural water. The data do not agree with the observations of Rice and Miscall, who reported that the solubility of copper at the boiling point of milk was less than at room temperature. It should be borne in mind, however, that these investigators exposed the metal for a long, continuous period, and therefore the change in composition of milk at the higher temperature undoubtedly became a decided influencing factor.

According to Hunziker, Dordes, and Nissen (51), milk is exposed to metal surfaces from the time of its production, through the various stages of handling and manufacture, to the packing of the finished product for market. Experience with the use of diverse metals in milk work has demonstrated marked differences in suitability of metals for dairy equipment. These experiences have shown convincingly that suitability of metals is not confined to those properties that deal with structural features, heat conductivity and durability, but that it demands serious consideration also of the relation of metals to the flavor and keeping quality of the milk product. In the study made by these authors, experiments were carried out on the action on aluminum and aluminum alloys, copper, nickel and copper alloys, iron, zinc, and galvanized iron, tin, tinned copper and tinned iron, 2 ordinary chromium steels, and 1 chromium-nickel steel of weak organic and mineral acids and of sweet and sour milks and creams, and the effect of the metals on the flavor of the milk products. Allegheny metal, tin, and heavily tinned copper were found to have no effect on flavor and showed maximum resistance to corrosion and tarnishing. The tin coating on copper must be heavy and intact. Nickel, aluminum, and manganese-aluminum alloy were found not entirely satisfactory in high acid milk products. Furthermore the nickel tarnishes readily and the aluminum is sensitive to alkali washing powders. Monel metal, Enduro, Ascology, and nickel silver gave the following results: Monel metal tarnishes appreciably in milk products but has only slight effect on flavor; Enduro and Ascology are sensitive to high-acid products, and their performance in dairy equipment is uncertain; nickel silver tarnishes severely and was injurious to flavor in the majority of milk products; tinned iron, copper, galvanized iron, and zinc were found unfit for use in contact with milk products, with the exception of properly tinned iron. Tinned iron is equally unfit whenever the iron is exposed.

Quam (52) determined the solubility in milk of the purest metals obtainable by the same methods previously reported by him and his coworkers. The curves for copper, nickel, and zinc were similar to those reported by Frazer, Ackerman, and Sands, who found a break at 70° C. in their curve for corrosion of Monel metal in aerated 5-percent solution of sulfuric acid. Quam stated that the break in the curve is probably due to the change in the solubility of oxygen at that temperature.

According to Gebhardt and Sommer (53) the harmful effects of dissolved copper in dairy products on their flavor and keeping quality is now quite generally recognized. Experimental results and practical experience have demonstrated that dissolved copper is capable of producing a "cardboard" or "cappy" flavor in fresh milk and cream and that dissolved copper is an important factor in the development of tallowy and, in some cases, fishy flavors in such products as butter, condensed milk, frozen cream, and whole milk powder. The solubility of copper in dairy products under various conditions therefore has direct practical importance.

Equipment constructed of copper, copper alloys, and tinned copper is used extensively in the dairy industry because of installations that were made before the deleterious effect of copper was recognized as it is at present and because of new installations in which such metals are used on account of relative cheapness, mechanical advantages in the working of the metals, and high heat conductivity. Such use of copper equipment, according to these authors, may not be disastrous in all cases because the rate of copper solution is variable. Accurate information is therefore desirable concerning the factors that affect the rate of copper solution, so that the conditions may be recognized under which copper equipment may or may not be used. The effects of acidity, dissolved gases, and temperature on the solubility of copper in milk were studied. The effect of velocity was not studied, but it was controlled and kept uniform in all the experiments. It was found that higher acidities markedly decreased (rather than increased) copper solubility in milk, while slight increases in acidity had no effect. The effect of dissolved gases was studied by bubbling them through the milk during the entire exposure. It was found that carbon dioxide markedly decreased, air slightly increased, and oxygen markedly increased the solubility of copper in milk. Prolonged treatment of the milk with oxygen before exposure led to the formation of a protective oxide coating. Temperature greatly affected solubility of copper in milk. The maximum solubility was always found at 70° C. whether the time of exposure was 30 minutes, 1 hour, or 2 hours. In high-acid milk the maximum was also at 70° C. but was appreciably lower than in sweet milk. At boiling temperature copper solubility was about the same as at room temperature. Up to 50° C. the temperature-copper-solubility curve followed the temperature-reaction-velocity curve. At higher temperatures, the curves diverged, less than the expected amount of copper being dissolved. Preheating milk to various temperatures above 70° C. decreased solubility of copper in such milk. The effect of preheating was greater as the temperature and time of preheating increased. The oxidation-reduction potential of milk was found to be related to the solution rate of copper and the various factors that affect it. Up to a temperature of about 65° C. the oxidation-reaction potential remained substantially constant; above 70° C. it decreased rapidly. With prolonged holding at 60° and 70° C. the potential remained constant. The presence of dissolved copper made the potential more positive. Increased acidity is known to make the potential more negative. Copper solution in milk is a typical case of metal corrosion. The same factors in the same relative order of importance apply to copper corrosion by milk as apply in general to metal corrosion by weakly acid or alkaline solutions.

In a succeeding paper, Gebhardt and Sommer (54) reported observations on the effect of acidity, of dissolved oxygen, of temperature, of steam sterilization, cumulative corrosion losses with intermittent exposure, and the type of tarnish developed. In the test for the effect of oxygen, this gas was bubbled through the milk at a temperature of 62.8° C., the usual pasteurizing temperature and also that of the maximum solubility for most metals. It was found that, in the presence of an increased oxygen content, the corrosion of all the metals increased with the exception of aluminum, which showed no perceptible corrosion in either case, and of tinned iron and stainless steel, which showed decreased corrosion in the presence of more oxygen.

In regard to temperature, it was found that the solubility of copper and copper alloys - brass, Monel metal, and German silver - increased markedly with the temperature up to a maximum of 70° C., beyond which the solubility sharply declined so that it was about the same at boiling temperature as at room temperature. The same was true of nickel, except that the maximum was found to be at 75° C. This point of maximum solubility differed appreciably from previous findings, and the difference was attributed to the lack of agitation in the work of Rice and Miscall and unusual opportunities for oxygen solution in the work of Quam. The solubility was so low and varied so little with temperature in the case of aluminum, Allegheny steel, stainless steel, and tinned copper that no definite conclusion could be

drawn as to the temperature effect. In the case of zinc, galvanized iron, and solder-coated copper there was a well-defined tendency to show a minimum solubility in the temperature range where copper, copper alloys, and nickel showed maximum solubility.

Tests on the effect of steam sterilization showed that copper oxidized by holding it momentarily in a Bunsen flame dissolved 3.5 times as much and copper oxidized by immersion in chlorinated soda solution dissolved 6 times as much as bright copper. Steam sterilization of the metal caused pronounced increases in the corrosion of zinc and galvanized iron, a slight increase in the case of copper, a slight decrease in the case of brass, and pronounced decreases in the case of Monel metal and nickel.

The metal test pieces were examined after each exposure to observe tarnishing. Of the metals studied, only nickel and Monel metal showed pronounced tarnishing in the submerged corrosion tests.

From the experimental results reported, it appeared that the question of the role of acidity in metal corrosion must be answered individually for each metal and for various temperatures; the effect of acidity is never as great as that of dissolved gases and temperature and, therefore, cannot be considered the chief factor in metallic corrosion in milk.

Ross (55), in a study of the development of a tallowy flavor in strawberry ice cream, found that oxygen absorption, iron, or strawberries did not develop the off flavor but that ice cream made with condensed milk developed the off flavor more readily than ice cream made with skimmed-milk powder. He concluded that the higher Cu content of the condensed milk, in conjunction with the oxidations of the fat of the condensed milk that occurred during manufacture and storage, aided in producing off flavors. It was suggested that the fat in condensed milk was carried virtually through the induction period of oxidation before it was used in ice cream and that relatively little further oxidation was necessary after the ice cream was manufactured for the development of the undesirable flavors.

Davies (56) found that small amounts of heavy metal are distributed between cream and separated milk in proportion to the curd N, but on complete separation of the fat from the skimmed milk a higher concentration of the metal occurs in the cream, showing adsorption of metal proteinate at the fat-globule surface. The heavy metal entering into butter from cream at different acidities is not affected by the acidity but by the curd content of the butter. The Cu associated with the curd in butter is approximately twice that associated with the protein N of the buttermilk. The entry of heavy metal into the fat phase in the absence of protein is greatest at neutrality, decreasing with increasing acidity. The amount of diffusible heavy metal increases directly with the acidity and runs parallel with the diffusion of H ions. The concentration of the ionic form of heavy metal in milk is of low order, but increases in acidity cause more ionic metal to form. Evidence from potentiometric measurements in milk containing known amounts of heavy metal or from the addition of milk to a standard Cu solution at various acidities confirm this finding.

Copper Content of Plants

The copper content of vegetables was first observed by John (57) in 1814-15 and communicated in a paper, which received a prize in Holland in 1816 but was not published until 1819. The first published statement on copper in vegetables was issued in 1816 by Meissner (58), who investigated a series of officinal plants.

Meissner (59) published a series of works on the existence of copper in a large number of vegetables from 1828-30. In 1830 Sarzeau (59) stated that copper could exist in organic materials or copper phosphate. In 1833 Boutigny (59) verified Sarzeau's results. In 1848 Deschamps (59) called attention to the presence of copper in the soil. Du Moulin (59) said that the toxicity of copper had been singularly exaggerated and defended the legitimacy of the introduction of copper sulfate into flour destined for baking. In 1883 Galippe (59) stated that, although the normal existence of copper in vegetables and animals, generally accompanied by iron, had been proved, it was not yet universally accepted. It seemed to him a sort of contradiction to admit, at the same time, the extreme toxicity of copper and its compounds and the presence of this metal in the vegetables growing around us, as well as in the animal and human organism. However, he said, it was true that copper was found universally in nature. He mentioned that Vauquelm discovered copper in a plant that he was analyzing, but it appeared to him so extraordinary that he neglected to call attention to the fact.

In 1893 Tschirch (15) said there was a possibility that all plants contained copper, as it had been found in many, that even cereals and other important foods and drinks contained not inconsiderable amounts of copper taken from the ground and that living plants absorbed copper through the roots as well as through the epidermis and always took it up when it was offered to them in the soil. He thought that it was really surprising that the human body did not contain more.

During the period 1914-26, McHargue (60) made investigations regarding the copper content of plants and developed a method for its estimation in plant and animal tissues. He found small amounts of copper widely distributed in rocks, soils, waters, plants, and animals, the largest amounts in the young and tender leaves and in the germs of the seeds of plants, and considered it a normal constituent of the blood and tissues of mammals, where it occurred in greatest concentration in the offspring before and at the time of birth. He found colostrum to be relatively rich in copper and normal milk to contain appreciable amounts (it occurs in the fat and most of that carried in normal milk is separated with the cream); upon churning the copper remains in the butter. When the butter is heated, curd is precipitated and carries with it considerable copper. The occurrence of copper in greatest concentration in some of the most vital organs of plants and animals led him to infer important biological functions for this element in the normal metabolism of plants and animals.

The presence of copper in the organism of vegetables was at first considered accidental, but according to Maquenne and Demoussy (61) it has since been recognized as normal for all species. An investigation was conducted to determine the proportion of copper in the most varied soils and if possible the influence it exerts on vegetation. They found copper everywhere, but in very unequal proportions, varying from 1 to 250 mg. per kilogram of earth. The metal, according to these authors, is retained by the fine particles of the soil due to their absorbent properties as well as to the chemical reactions that may occur there, and only an infinitesimal proportion is carried away in drainage water. In a soil containing 50 mg. of copper per kilogram the drainage water contained only 0.03 mg. per liter, an amount incapable of exercising any harmful action on vegetation in the presence of lime, which by its stimulating action is the best antidote for copper. It is due to this capacity of absorption that the copper never descends into the deep layers of the soil. The reason given for the innocuousness of the extended use of copper, so very toxic by itself, on the soil is that the absorbent properties of the soil retain the copper and immobilize with an energy that the plants cannot conquer, therefore there is nothing to authorize the supposition that this metal made insoluble will some day be redissolved in disturbing amount. The following statement is made in regard to copper in vegetables:

It has been known for a long time that vegetables contain copper but absolutely nothing has been known of its division in the different organs of the same plant, due to the fact that the analytical methods in use do not lend themselves to the very great number of observations necessary to solve this question. By the method used, a very great number of facts were assembled. Copper was found in all parts of the same plant, from the root to the stems, leaves, flowers, and fruits; this indicates a mobility that was not known in regard to copper and which is very similar to that possessed by other metallic substances, such as lime, manganese, and potash. This faculty of migration pertains only to substances soluble in cellular liquid.

The results are especially suggestive from the point of view of distribution of copper in the different organs of the plant, which can be summarized by saying that always copper shows its preference for the parts where vital activity is or is to become the greatest. At the end of spring more is found in the stems than in the wood; the difference is accentuated ordinarily to the degree that the vegetation becomes more active and the young leaves growing from these stems contain more copper than all the other parts of the plant. The leaves of evergreen species of one year are much richer in copper than those of the preceding year, as the latter have given up their metal to satisfy some physiological need of those which must replace them; the same thing is observed with the phosphates and protein substances. At the approach of maturity, the copper turns completely toward the fruit. With the legumes, the peas or kidney beans, the seed, still green contains almost as much copper as the pod; at maturity when all is dry, the seed contains more. The seed itself, decorticated, contains more copper than its teguments, the kernel of a nut more than the shell. The falling of the fruit is evidently one of the modes of elimination of the copper the plants have absorbed during the course of their active life.

These determinations prove that copper is not disposed in the vegetable tissues in the manner of an accidental element by simple evaporation of the water which introduced it. The mode of distribution of copper is just exactly the reverse of that of lime, which is always more abundant in the old organs, as the dead leaves, the bark, or even the pod of the legumes which have evaporated much water, than in the young organs or even the matured seeds. The transportation of copper toward the vital parts of the plant is then a physiological process analogous to that which occurs with the migration of the principal essentials of nutrition, nitrogenous materials, phosphoric acid, and potash.

These researches have thus aroused anew this question, which in spite of its high physiological interest, has never been scientifically solved: Is copper necessary, favorable, or only indifferent to the development of plants? It is generally considered as an accessory of no effect if its proportion does not reach the point where it becomes toxic.

It was concluded from this study that copper, in a suitable dose, is an essential migratory element that is replaced in the plant from time to time and in the same sense as its principal foods without harming in the least the tissues through which it passes.

According to Bertrand (62), long and difficult investigations have disclosed that the bodies of human beings, animals, and plants contain in the normal state minimum amounts of the following metalloids and metals: Metalloids - arsenic, boron, bromine, iodine, and silicon; and metals - aluminum, copper, manganese, vanadium, and zinc. The reason these 10 elements have so long escaped the investigators is because they are present in extraordinarily small amounts and methods of analysis have not been sensitive enough to discover them. A list of vegetables and fruits is given with the amounts of copper in milligrams per kilograms of fresh material. The question is raised as to whether these substances are physiological elements or whether their presence is merely accidental. In Bertrand's opinion they are physiological, but enough investigative work has not yet been done to determine this question. He mentions the role played by iron as a conveyer of oxygen in the blood and calls attention to the remarkable fact that copper replaces the iron of the blood of vertebrates in the crustaceous animals and the molluscs. In the organism, animal or vegetable, almost all, if not all, the chemical transformations are conditioned by catalytic reactions, which he calls diastases. Contrary to the industrial catalysts, the diastases are organic in nature and possess a very complex composition. The metals intervene as catalyzers in the same capacity as the diastases produced by the organism and at times not always in union with them.

Guerithault (63) mentions the fact that the question of copper has long attracted the attention of the analysts, and several of the scientists have investigated copper in living beings, but he is giving in this note the results of his own experiments on the amount of copper in vegetables. The method of determination by incineration, treatment of ashes with hydrochloric acid, separation of the copper in the form of sulfur, reduction of the sulfur to nitrate, electrolysis, and, finally, determination of the amount of copper by direct weighing or by colorimetry after addition of nitric solution of ammonia was used. The copper content of 44 vegetables, fruits, and nuts was determined. The proportions found in Guerithault's experiments varied from 8.7 mg. copper in radish seeds to 63.6 mg. copper in sweet almonds, per 100 gm. of ash. The seeds were found to be particularly rich in copper.

In a second article McHargue (59) discussed further the significance of copper in plants as follows:

It has been recognized for some time that yellow corn possesses a slightly higher food value for feeding livestock than white corn. The difference in the copper content of the white and yellow corn grown upon the same type of soil during the same season is of particular significance from the standpoint of food values. This significance finds support in the copper content of the foliage of red clover and alfalfa, which are recognized sources of important vital factors in the nutrition of domestic animals. It is a well-known fact that the germs of corn and other cereals contain important vitamin factors, whereas the products made from the endosperm after the germ and bran have been removed in the modern milling processes are deficient in the vital factors. The further association of copper, manganese, and zinc with vital factors is shown in the analyses of the highly milled products of wheat and rice. Wheat bran is relatively rich in copper, manganese, and zinc and the germ is particularly rich in copper and is also relatively rich in manganese and zinc, whereas in the highly milled patent flour most of the mineral matter has been removed in the offal. The germ of wheat is regarded as a rich source of an unidentified vitamin factor. The association of relatively large amounts of copper, manganese, and zinc with a substance rich in

vitamins is a coincidence of striking interest. It is also to be observed that when rice is polished nearly all the copper, iron, manganese, and zinc are removed in the polishings. Consequently, when pigeons are confined to a diet of polished rice they soon develop polyneuritis, whereas pigeons fed on unpolished rice maintain a normal condition.

Authorities on the subject of vitamins assume that these important factors are synthesized in plants and not in animals. From the results contained in this paper, plants absorb small amounts of the elements copper, iron, manganese, and zinc from the soil and from them synthesize compounds which are not poisonous but apparently are vital factors in plant and animal metabolism. If this assumption is correct, then copper, manganese, and zinc perform more important functions in agriculture than is generally recognized.

McHargue (64) determined the mineral constituents of Kentucky blue grass at a stage in its growth when best suited for grazing. Analysis showed that 0.0014 percent copper was found in the moisture-free young blades and 0.019 percent in the ash; 0.032 percent iron was found in the moisture-free blades and 0.412 percent in the ash; 0.008 percent manganese and 0.017 percent zinc were found in the moisture-free blades, and 0.115 percent manganese, and 0.221 percent zinc were found in the ash. Data presented previously by McHargue showed that more than the 10 elements - carbon, hydrogen, oxygen, nitrogen, phosphorus, calcium, magnesium, potassium, iron, and sulfur, the so-called essential elements - are necessary for the normal growth and maturation of plants. According to him, evidence has been obtained that manganese is an essential element for the growth of plants and performs an important function in the synthesis of chlorophyll, and results recently obtained show that small amounts of copper have an additional beneficial effect. Three plants of the same age were fed as follows: 1 received the so-called 10 essential elements only, 1 received manganese in addition, and 1 received both manganese and copper besides the so-called 10 essential elements. The difference in growth attained by these plants led McHargue to conclude that copper as well as manganese has an important function in the growth of plants and that these 2 elements, manganese and copper, are 2 of the vitamin factors that Harrow suggested may be necessary for their growth. The largest percentages of copper, manganese, and zinc occur in the seed. The same relationship has been noted in a number of other plants. In seeds that have germs large enough to permit of dissection from the endosperm - wheat and corn, for example - it has been shown that most of the copper, manganese, and zinc occurs in the germs and not in the endosperm.

McHargue (65), after considerable experimenting and study, is more and more convinced of a vitamin cycle in nature in which small amounts of the elements copper, manganese, and possibly zinc, nickel, and boron, are absorbed from the soil by plants and with the aid of these elements plants synthesize complex organic combinations that play an important function as catalysts, enzymes, and vitamins. When these organic compounds, which have been synthesized by the plants, are consumed by animals they are assimilated and resynthesized into catalases, oxidases, and hormones, or animal vitamins, which function in the excretions of the various organs in a more concentrated form. When the food consists of adequate amounts of green vegetables, eggs, fresh meats, including liver, fresh whole milk, and whole and unaltered cereals, the human body will obtain from such foods all the organic metal vitamin complexes, from which it can resynthesize its own organic vitamin complexes to meet its own peculiar needs and requirements.

In 1931 Cunningham (66) wrote that, within recent years, our conception of the mineral nutrition of animals has had to be broadened to take account of certain "trace" elements that have been found to be widely distributed in the vegetable and animal kingdoms. These elements (for example, manganese, iodine, zinc, and copper) occur only in minute traces, but their possible physiological significance cannot be neglected. The occurrence of copper in the vegetable and animal kingdoms, copper and iron metabolism, and the toxicity of copper to animals were discussed by Cunningham. He found more copper in the aerial parts of plants than in the roots but concluded that this is probably not connected with chlorophyll content as the white inner leaves of cabbage and lettuce contain as much (or more) copper as the outer chlorophyll-rich leaves. The stems and pods, where a large amount of fibrous material is to be found, contain less copper.

Remy (67) considered of real significance the finding that the vitamin content of preserves apparently stands in reverse relation to that of the amount of copper naturally present in them. The highest content of copper was determined in apricots and the lowest in strawberries. Vitamin C is always accompanied by a reducing salt.

In the report of the Committee of the Food and Nutrition Section of the American Public Health Association (68) given at the meeting in 1932, attention was called to the importance of fruits and vegetables in providing dietary essentials, as revealed by the recent research appearing in the scientific press. According to the report, relatively few of the data are old enough to be in the standard textbooks on nutrition or dietetics and much of it is 1932 research. An article by Hodges and Peterson, published in 1931, is quoted as saying that an ordinary serving (1/2 cup) of cooked cranberries provides 0.15 mg. of manganese, 0.05 mg. of copper, and 0.30 mg. of iron. Any attempt merely to list the new facts about common fruits and vegetables in 1931-32 research articles, according to the committee, would expand their report to a volume of encyclopedic size. Among the values listed by the committee as contributions to be expected from this group of protective foods in securing what "McCollum happily describes as the preservation of the characteristics of youth" was that of especially important sources of the antiscorbutic vitamin C, and the hemopoietic elements iron and copper.

Copper Content of Animals

According to Lehmann (58), the high copper content of numerous snails and mussels (especially the respiratory organs) was first discovered in 1833 by Bartholameo Bizio, who made quantitative determinations; the ash of *Spira of Murex brandaris* yielded 4.2 percent copper oxide, 1.55 percent in the mucous membrane, and only 0.01 percent in the muscle.

Harless (69) detected copper in the blood of *Eledone* and *Helix pomatia* in 1847 and showed that it existed not as a free salt but in combination with protein. This peculiar copper-containing protein was named hemocyanin by Fredericq (70) in 1878. In 1885 Halliburton (71) wrote as follows on the blood of Decapod crustacea:

Hemocyanin is the only proteid hitherto described in the blood of crustacea. It is an interesting substance occurring pretty widely distributed in various classes of invertebrata. It always contains a small quantity of copper, which seems to take the place of the iron of hemoglobin. A good many different observers described the blue color and the presence of copper in the blood of various animals, but it was not until after Fredericq made his observations on the blood of the octopus and proved the existence of this body to which he gave the name of hemocyanin, that it was possible to draw comparisons between the bloods of these various animals.

Harless showed that the blue coloration in the *Eledone*, *Sepia*, *Cancer pagurus*, and the *Helix pomatia* was the effect of exposure to the atmosphere and he also showed the presence of copper in the blood and a trace of iron.

Genth showed that the blood of *Limulus* assumed in a similar way a blue tint on exposure to the air, and that it contained copper and a small amount of iron.

Gorup-Besanez added *Acanthias* and *Unio* to the list of animals containing copper.

Fredericq showed that the addition of hydrochloric acid to hemocyanin splits up that body into two products; one of these is a coagulated proteid containing no copper, and the other a colorless solution of a substance containing copper. Fredericq describes copper as occurring in pure hemocyanin but not iron. I confirm his observations in this. Hemocyanin is a globulin. It differs from serum globulin in certain points: one difference is that it contains copper while serum globulin does not.

Griffiths (72) found 0.18 to 0.24 percent copper oxide in the ashes of blood of 10 different species of invertebrata. He (73) found in the hemocyanin of crustacea (*Homarus*, *Sepia*, and *Cancer*) 0.31 to 0.36 percent of copper in precipitated and dried hemocyanin. Hemocyanin, according to him, is very uniform in composition and much more stable than hemoglobin.

Herdman (74) found the quantity of copper present in oysters of different origin to be fairly constant, varying from 0.25 to 0.66 mg. per oyster, or from 0.30 to 1.18 percent of the ash. The average of copper (0.4 mg. per oyster) is slightly greater than the average for iron (0.26 mg.). His results show that copper is present normally in both green and white oysters in small quantity but that the greenness of the French oyster is certainly not due to this metal. However, the green color of diseased American oysters was found to be coincident with the excessive quantity of copper present.

In regard to hematic copper of invertebrates and the respiratory capacity of the hemocyanin, Dhéré (75) wrote as follows:

Leon Fredericq supposed that the combination of hemocyanin with oxygen is formed in the presence of the copper in the molecule of this substance. This hypothesis is justified by the analyses of Harless and von Bibra, Witting, and Genth, who revealed the existence of copper in the blood of various mollusks and crustacea, and seems to be affirmed again by the subsequent works of Krukenberg and Griffith. However, Heim, from a study on the hemocyanin of crustacea Decapoda, arrived at the conclusion that copper could not have entered into the composition of the pigment because it was absent from the blood of the spiny lobster, turtle, and crawfish.

Dhéré himself made a study and found that copper is a normal constituent of the hemolymph of the snail, the poulp, the turtle, the lobster, the spiny lobster, and the crawfish but that the proportion is rather variable from one species to another and from one individual to another of the same species. These variations seem to parallel the variation of intensity of the blue color of the blood, at

least in one species given. Another justification called for by the theory of hemocyanin is that of the proportion of the content in copper of the blood to its capacity to absorb oxygen.

According to Henze (76), the blood of Cephalopoda (octopus, eledone, sepia) is absolutely free of iron and contains as albuminous substance the copper-containing hemocyanin. He found in the liver of the Cephalopoda copper varying in amount from 0.19 to 0.762 percent. The copper content was found to be 10 times that of the iron. It was desired to find out with what the copper is combined and in what form it is present, so Henze turned to two different liver pigments, determined by Dastre and Floresco in the octopus, one a water-soluble iron-containing pigment the other insoluble in water but soluble in chloroform and other organic solvents, and analyzed them. Analysis of the chloroform-soluble pigment revealed not the smallest amount of copper or iron. Analysis of the water-soluble pigment after ashing gave strong reaction for copper and phosphoric acid. From the results obtained, Henze doubted that he was dealing with constant compounds. He analyzed the pigment from four different animals with the following results: Pigment I, copper content 3.55 percent; pigment II, copper content 7.77 percent; pigment III, copper content 6.71 percent; pigment IV, copper content 1.29 percent.

Bradley (77) found traces of copper, estimated electrolytically, in the blood of *Fulgur carica* and in amounts varying from 7.83 to 8.57 percent in the blood of *Sycotypus canaliculatus*.

According to Rose and Bodansky (78), the more active species requiring a rapid respiratory exchange are provided with blood containing larger percentages of copper than other species of a low metabolic type. They quote Dhéré as finding the efficiency of a hemocyanin as an oxygen carrier to be directly proportional to its copper content, which is of considerable physiological interest. Because of the incompleteness of the data on the occurrence of copper and zinc in many forms of marine life, Rose and Bodansky made a quantitative study of their copper and zinc content, confining their work chiefly to fishes. A colorimetric method, based upon the "blue test" with an excess of ammonia, was used. Oysters were found by far the richest in copper of the animals examined, the content varying from 24 to 60 mg. per kilogram. The copper was found rather uniformly distributed throughout the tissues, except that only half as much occurred in the muscle as elsewhere in the organism. Part of the copper in oysters (12.2 percent in these experiments) exists in a diffusible form. Analysis of 20 specimens of common fishes showed the presence of copper in every case except 1, and the failure to detect it in this case was probably due to the small amount of material available for analysis. The average copper content was approximately 2.5 mg. per kilogram. Copper is believed to be a normal - possibly an essential - constituent of marine animals, and not an accidental one due to the trace of this metal in sea water.

Alsberg and Clark (79) studied the hemocyanin of *Limulus* and compared their results with those of Henze on octopus. The hemocyanin of *Limulus* was found to differ from that of octopus in percentage composition, in its precipitability by dialysis, by full saturation with magnesium sulfate, and by half saturation with ammonium sulfate, in not having been crystallized, and perhaps in being more sensitive to acid. There are, therefore, different hemocyanins, and perhaps this fact accounts for the discrepancies in the literature concerning the properties of this substance. Copper was determined by incinerating in porcelain and exhausting the ash with nitric acid. The acid solution was evaporated to dryness with a little H_2SO_4 . The solution, taken up in a little very dilute H_2SO_4 , was filtered and the copper determined electrolytically; 0.515 gm. of substance yielded 0.0015 gm. Cu; Cu = 0.29 percent. Henze obtained an average of 0.38 percent copper from octopus blood. The difference in the copper content is considerable. Perhaps it may be

found that animals more sluggish than the octopus possess hemocyanins of lower copper content, and therefore less active oxygen carriers. Certainly, according to Alsberg and Clark, their blood as a whole usually contains less copper. Like Henze, Alsberg and Clark were unable to obtain any copper compound analogous to hematin. They regarded their substance as a copper globulin compound, whereas Henze regarded his as a copper albuminate. They are probably two different proteins.

Dhéré (80) found the existence of a roughly proportional relation between the content of copper and the greatest volume of oxygen absorbable by the bloods of mollusks and crustacea. The relation is fairly constant, but for the same content of copper the blood of the crustacea fixes more oxygen than the blood of the mollusks. Dhéré stated that his findings agree with those of Winterstein.

The content of copper in both fresh-and salt-water fish, as determined by Parks and Rose (81), averaged about 2.5 mg. per kilogram of moist material. Salt-water fish contained about 12 percent more iron than fresh-water fish. Species of fish with dark tissue contained approximately 75 percent more iron than the species with light tissue.

According to Lehmann (58), the first definitely to determine copper in general in a warm-blooded animal appears to have been Sarzeau in 1830 and 1832 when he found 3 mg. of Cu in 4,286 gm. of flesh and blood of the meat. Devergie (58) in 1840 was the first to report what Deschamps had suspected, the presence of copper in human organs. At the same time Orfila (82) also found copper in small quantities normally in all tissues of the human body.

According to Galtier (14), Devergie recovered copper from the intestinal tract of three cadavers; struck by this coincidence, he undertook some special investigations with Hervy, who pointed out the presence of lead also. After that Devergie made no legal analysis without meeting these two metals in all the organs, especially in the intestinal tract. He found that the relative proportions varied according to age, nourishment, and morbid condition; less in the newborn, 4 or 5 times greater in the adult; lower in the young and in the ill, which led to the thought that it provided alimentary material. Although he found that the proportion in the intestinal tract of adult men and women varied, it did not exceed 46 mm. for copper and 40 mm. for lead. In every case the copper surpassed the lead, if there was no lead encephalopathy. Orfila (82) in 1838 made a large number of experiments, which confirmed the results of Devergie, after which he admitted the existence of normal or physiological copper. Cattel and Platner (83) in 1840 were unable to find, by incineration, copper and lead in the digestive tract, heart, lungs, liver, and kidney of several day-old infants; they concluded that this metal did not constitute a part of the organs and that its presence was only accidental. Flandin and Danger (84) in 1843 denied not only the normal copper but even the presence of accidental copper and lead, not having been able to recover copper by their process from the bones, muscles, viscera, and urine of animals not poisoned. Their results were contrary to the results obtained by Louis, Orfila, Millon, and Laveran. Barse, Follin, Laneau, Chevallier, Lassaigne, Payen, Legrip, Deschamps, and others (85), with repeated experiments and at different times, also obtained copper from the organs of persons not poisoned.

From the preceding facts, Galtier (14) concluded that, among the toxicologists, Orfila, Millon, and others considered the presence of copper and lead as the normal or physiological condition, in which Devergie seemed to concur. On the contrary, Flandin and Danger denied the normal lead and copper; Galtier agreed with Barse, Chevallier, and Chatin that these metals were met with constantly in the organs but that they arrived there accidentally by the intermediary of food and the copper and lead vessels so frequently used. In a case of suspected poisoning by copper,

Chevallier, Devergie, and Payen, having obtained about 15 mg. of copper (an amount that could be considered as normal or accidental), analyzed comparatively the same quantity of liver and intestine of a person aged 32 who had drowned and obtained almost the same amount of copper. Not knowing the antecedents, they concluded that the quantity of copper found in the suspected case did not necessarily presuppose copper poisoning. Galtier (14) reported the case of a girl whose parents were accused of having poisoned her. Autopsy revealed copper in the various organs as follows: 0.274 gm. of copper in 250 gm. of intestinal tract; 0.091 gm. of copper in 27 gm. of excremental material; 1.170 gm. in 100 gm. of liver; the heart, lungs, stomach, kidneys, and muscles furnished a more or less large quantity of copper. The defendants were acquitted on the ground that the copper was physiological.

In 1877 Raoult and Breton (86) presented to the French Academy of Sciences the results of a judicial examination made at the close of 1874 at Grenoble, as it was believed of value on account of the renewed agitation among toxicologists of the question of the occurrence of copper in the body of man. They stated that the proportion of copper and zinc, called normal, existing in different individuals probably varied greatly according to age, state of health, nature of food, and the ordinary utensils in contact with their food and drink. Therefore, in order to have chemical proof of poisoning by the compounds of copper and zinc, it is not enough to determine the existence of these metals in the cadaver; it is necessary to determine, in addition, that the quantity of copper and zinc found in a given weight of incriminating material is considerably higher than the maximum quantity found by the same methods in the same weight of other materials obtained from persons having lived under nearly the same conditions. The authors summarized the results of their analyses in the following table:

Organ	Copper	Zinc
	Mg. per kg.	
Intestine of drowned person	Trace	None
Liver of a calculous person	3	10
Liver of a consumptive	15	30
Liver of a young woman	7	34
Liver of an old man	10	76

Gienandt (87) found in guinea pigs that had been fatally poisoned by 0.024 to 0.03 gm. copper in the form of Harnack's solution very little copper in the individual organs, which was to be expected since the fatal dose was so extremely small. He stated that, with general copper poisoning, the copper is found constantly in the stomach, kidneys, and blood.

Occasionally, from investigations carried out on the character and cause of the so-called brassfounders fever, Rost (88) found measurable and constant amounts of zinc and copper in the feces and in the urine when the total amount was subjected to chemical analysis. In all cases he found a smaller amount of copper than of zinc. He considered the finding of zinc and copper for more than a week after the subjects had left the foundry to indicate that, aside from the temporary inhalation and absorption of these metals from the foundry fumes, there must be still another permanent source for these constituents of the feces. He concluded that these amounts of metal were due solely to the daily food. In one case only 119 mg. of copper was found in cattle liver; 4 mg. were found in lake fish and 0.5 mg. in 1 egg. These findings he considered important, not only for the investigator but also for the legal chemist and the food chemist. He said it was for physiology to determine whether these amounts of zinc (and also copper) have any significance of

importance to life, like iron. He decided from his investigations that, under present living conditions, zinc and copper are accidental but ordinary constituents of human, animal, and plant substances and that neither physiological nor hygienic significance is to be attributed to their presence. He said these extremely small amounts were not serious for human beings from the health standpoint and that the earlier anxiety about copper had completely disappeared.

The presence of traces of copper in the human brain was first reported by Thudichum in 1901, according to Bodansky (89). Bodansky analyzed four adult brains and a fetal brain; the values found for copper fell within the limited range of 3.6 and 6.8 mg. per kilogram. He concluded that the results indicate that copper and zinc are normal constituents of the human brain; judging from the one fetal brain, it appears that during intrauterine life there is more rapid storage of zinc and copper in the brain than there is after birth. In this respect the behavior of these elements is similar to that of other inorganic constituents of animal tissues such as iodine, sulfur, and phosphorus.

McHargue (90) said that the occurrence of some of the so-called nonessential elements in Kentucky soils and in certain tissues of plants and animals produced on such soils and the fact that their concentration is greatest in certain vital organs of plants and animals is a coincidence that suggests the possibility that such elements may have important functions in the life processes. Copper was found in animals substances in the following amounts, expressed in parts per million of moisture-free material:

Animal and organ	Copper content, parts per million
Lean meat of ox	0.4
Liver of same ox	75.0
Liver of week-old calf	345.0
Blood of same calf	8.0
Testicles of mature horse	154.0
Egg yolks	2.5
Cod-liver chum	44.0

The fact that the liver of a young calf contained so much copper is both interesting and significant in the author's opinion. As the calf was only a week old it must have obtained all this copper from the mother cow. This raises the question as to the effect on the offspring if the food of the mother cow had been deficient in copper, manganese, or zinc.

The accumulation of copper in greater concentration in the fetuses of mammals is strong evidence, according to McHargue (60), that it performs important functions during development of the embryo in the early stages of growth after birth. Its occurrence in the yolk of eggs is further evidence of important biological functions in development of the embryo. The liver is an organ in which important biological oxidations and transformations are constantly taking place. It has been demonstrated that the tissues of this organ contain oxidizing enzymes and that it exhibits respiratory properties to a remarkable degree after removal from the animal body, whereas the juices that may be pressed from it have little, if any, respiratory properties. It therefore is reasonable to assume that the copper in a normal liver is concerned in the catalytic transfer and oxidative changes that take place in this organ. The fact that the tissue of the liver and the crude black oils prepared from the livers of codfish have a much greater fat-soluble A potency than

the highly refined cod-liver oil is evidence that supports the idea that fat-soluble A is probably a colloidal compound of copper that occurs in the liver of animals to the greatest extent but has been eliminated to a large degree in the highly refined cod-liver oil. The same explanation is to be offered in the occurrence of copper in the cream of milk and its subsequent occurrence in butter as it is ordinarily prepared for food. From the results contained in this and a previous report, McHargue was led to infer that an organic colloidal compound of copper, such as probably exists in green leaves, germs of seeds, whole milk, blood, livers, and the yolk of eggs, may be the fat-soluble A factor and that a similar compound of manganese in some of these materials may function as the water-soluble B factor.

Zondek and Bandmann (91) found that marked parallelism exists between the concentration of copper and that of vitamin B occurring in various sources.

In his textbook on chemistry published in 1875, Porter stated that copper is a normal constituent of human blood; but McHargue, Healy, and Hill (92) in 1928 said that, until presentation of their work, it had not been demonstrated that copper has an important function in red-blooded animals. They carried out an experiment to ascertain the copper content of the serum and cells of fresh blood of a normal 2-year old heifer that was slaughtered for beef; 0.0044 percent of copper was found in the dry serum and 0.0007 in dry cells and fibrin. Also 0.0125 percent copper was found in moisture-free calf liver. To demonstrate whether or not the copper in red blood has any useful function, as its presence suggests, 2 solutions of livers of young calves were prepared, 1 without and the other with copper. One set of albino rats was fed skimmed milk with addition of the liver solution containing copper and one set with the addition of the liver solution without the copper. When samples of blood for the hemoglobin determinations were taken, it was observed that the blood from rats from which copper was withheld was noticeably darker in color than the blood from the rats that received copper. The rats that received copper in their diet also possessed to a noticeable degree a pink ruddiness, where the skin was exposed, that was absent on those from which copper was withheld. From the external appearance of the rats, the color of the fresh blood, and the hemoglobin readings, it was inferred that copper has an important function in the formation of hemoglobin and in the metabolism of animals having red blood.

In view of the attention given to the presence of small amounts of certain elements in the animal organism, it was of interest to Hendrix (93) to study the concentration of copper and of hydroferrocyanic acid on coagulated protein from very dilute solutions. Careful attention was paid, not only to the actual amount of copper and of iron combined with 1 gm. of the protein, but also to the ratio between the amount of copper or iron combined with 1 gm. of the coagulated protein and the amount in an equal quantity of the solution. The amount of copper taken up by coagulated egg albumin increases with the pH at least up to the pH of 6.9. Preliminary studies indicated that the coagulated protein takes up the acid-radical ferrocyanide in acid solution, and the amount of ferrocyanide combined increases as the pH decreases to the pH of 3.7.

In 1928 Abderhalden and Möller (94) analyzed, by their own methods, serum from horse blood for iron, copper, and manganese content. The amount of copper found agreed, in order of magnitude, with that determined by Warburg for serum of human blood. Amounts of copper were found up to 2.64 mg. CuS or 2.11 Cu in 1,000 cc. of serum. Only very small amounts of copper and iron were found by dialysis. The findings brought up the question as to whether the metals mentioned are not contained in the form of ions in the serum. The phenomena of absorption by the serum proteins might injure the capacity for dialysis. It appeared that, in addition to the iron, copper was also contained in organic combination in the serum.

In a previous report, Krebs (95) had stated that copper appears constantly in human blood serum, the serum of human beings containing almost as much copper as iron, that is, about 1 to 2.10^{-3} mg. per cubic centimeter. In a report published in 1928 Krebs gave the results of his studies on the variation of the copper content in the blood serum in different persons. The cystein method was used for determination of the copper. The value in healthy fasting persons, independent of sex or age, was found always about the same order of magnitude, that is, between $0.62 \cdot 10^{-3}$ and $1.24 \cdot 10^{-3}$ mg. per cubic centimeter of serum, the average being $0.91 \cdot 10^{-3}$ mg. in cubic centimeter. The percentage variations with different persons were relatively small and about comparable with the percentage variations of blood sugar with fasting persons. The copper content of the blood serum of 26 cases suffering from various diseases (of metabolism, circulation, stomach and intestinal tract, carcinoma, anemia) was found to be within the normal limit. The average for the 26 cases was $0.94 \cdot 10^{-3}$ mg. copper per cubic centimeter. The copper values of the blood serum of 16 pregnant women were found to be increased, the average being $1.94 \cdot 10^{-3}$ mg. per cubic centimeter, that is, more than double the copper value of normal serum. Not only was it found that the copper content of blood serum increased in pregnancy but also in various diseases, such as tuberculosis of the lungs, acute infections (diphtheria, scarlet fever, endocarditis), leukemia, and bronchial asthma, the values varying from $1.04 \cdot 10^{-3}$ to $2.56 \cdot 10^{-3}$. A decrease was found in nephretic kidney diseases, the average of three cases - $0.20 \cdot 10^{-3}$ - being a fourth of the normal value.

Because no studies had been made directly on the presence of copper in pure hemoglobin, although the presence of copper in the blood had been reported by a few workers, and because the possible presence of copper in the molecule was the first question to be answered in determination of the function of copper in hemoglobin building, an analysis of this pigment was undertaken by Elvehjem, Steenbock, and Hart (96) in 1929. They found that, if the molecular weight of hemoglobin is accepted as 16,700 or 66,800, as reported by Svedberg and Fahraeus, the hemoglobin of rat blood does not contain copper as part of its molecule. The copper content of horse blood was found to be approximately 0.05 mg. of Cu per 100 cc. of blood. The corpuscle fraction of blood, whether prepared by centrifuging oxalated blood or defibrinated blood, was found to contain the largest portion of copper.

In 1929 Flinn and VonGlahn (97) examined 20 specimens of liver obtained from routine necropsies for copper content, which was found to vary from 2.30 to 12.42 mg. per kilogram; 2 other specimens were examined from workers in copper industries, 1 in a copper mine for more than 14 years, the other in a brass foundry for several years. It was interesting to note that the content of the livers from the workers in the copper industries was close to the minimum. The copper content of the livers of various animals was also determined and found to vary from 1.12 mg. per 100 gm. of liver in the rabbit to 15.6 mg. in the goat.

Kleinmann and Klink (98) in 1930, by a slightly modified form of the colorimetric method of Schonheimer and Oshima, determined the copper content of 12 normal adult livers, which they found varied from 11.8 to 48.7 mg. copper, with an average of 27.5 mg. These figures are slightly higher than those of Schonheimer and Oshima, according to Kleinmann and Klink. Two samples of blood contained, respectively, 1.7 and 1.4 mg. copper. A sample of cardiac muscle contained 2.2 mg. and one of skeletal muscle, from a younger person, contained 1.8 mg. The copper content of the liver in a typical case of hemochromatosis was 133 mg., a decided increase above the normal average. The liver in another case considered to be hemochromatosis, but with the pigmentary changes limited chiefly to the intestine, contained 18.3 mg. of copper. Two livers with nonpigmented cirrhosis contained, respectively, 8.7 and 3.9 mg. of copper. The relatively large amounts of copper found in the liver of fetuses and newborn infants was considered to have

greatest interest. The copper content of 10 such livers varied from 137.5 to 450 mg., with an average of 303 mg. The copper content of the livers of 3 children, aged 13 weeks, 14 months, and 2 years, varied from 12 to 26 mg. The thoroughly transfused liver of a newborn infant contained 516 mg. of copper, proving that the high copper content of the liver at this period of life is not due to the copper of the blood in the organ. The large amount of copper in the liver of the fetus and of the newborn infant is interpreted as evidence of the important functional role of this element in the normal physiology of the fetus and of the very young infant.

Mandai in 1931 (99), by the polarographic method of microanalysis, found the copper content of the liver of human beings to be 0.008 ‰, a little greater than previously reported. In the embryonic liver the mean copper content was 0.047 ‰, about 6 times as much as that of the adult liver. In the animal livers the copper content was 0.003 to 0.032 ‰.

Cunningham (66) stated in 1931 that a considerable number of copper determinations had been made in the case of marine animals, in which it had been shown that of 35 species the copper content varied from about 60 mg. per kilogram dry matter in the oyster to traces in the whale, the amounts diminishing progressively in ascending from the comparatively simple to the more complex organisms. He said the presence of copper in marine animals had been explained only in those whose blood contained hemocyanin.

With land animals, only in the case of the rat is there reliable information regarding the distribution of copper throughout the various organs of the body, according to Cunningham. Although some figures are available for the different organs of man, the ox, and the horse, the data are scanty, and the practice of taking different organs from separate animals does not permit definite conclusions to be drawn regarding the distribution of copper throughout the body. Cunningham determined copper in the organs from 12 different species of animals in milligram per kilogram of dry matter as follows: Liver, from 9.2 in the adult rabbit to 470 in the newly born calf; heart, from 12.8 in the adult badger to 27.8 in the rat; lungs, from 2.4 in the domestic adult fowl to 916 in the adult sheep; spleen, from 2.9 in the adult bovine to 8.1 in the rat; kidney, from 8.5 in the bovine fetus to 22.6 in the rat; 17.5 mg. copper were found in the brain of a human adult, 8.5 mg. in the brain of a 9-day old dog, 14.6 in the brain of an adult cat, and 108 in that of an adult badger. Although in all the organs so far dealt with the variation of copper content was too great to assign a definite value for it in a given organ, at the same time the differences between the various organs were significant, and the distribution of copper throughout the organs was substantially the same for all species examined. Classification of organs according to descending value for either proportion of copper to total weight of copper in the organ gave liver, kidneys, heart, lungs, pancreas, spleen.

According to Cunningham's results, copper is present in all parts of animals (and of plants) examined, and he thought it possible that this element may fill a small corner in the composition of protoplasm. A notable feature, according to him, is the high proportion of copper in sheep's liver; the average copper content of 9 livers was 323 mg. copper per kilogram dry matter, the range being 212.5 to 497.5. The high proportion of copper in livers generally might be due to infiltration from the blood of copper absorbed in the digestion of foodstuffs.

In 1923 Nitezesco (100) was led to study the copper content of the organs of the human fetus by the blue color of the flame when he was incinerating a piece of liver of a child that had died during full-term delivery. He obtained material from obstetrical clinics, accepting only infants from healthy mothers and infants that had died during delivery from accident, as poor position or asphyxia of cord. For

comparison he analyzed organs of adults obtained at autopsy in accidental deaths. He found an average of 26.2 mg. of copper per 1,000 gm. of fetus material and an average of 4.5 mg. per 1,000 gm. of adult material. His results showed that copper is not increased with age and that the liver of the newborn is much richer in copper than that of the adult.

Guillemet (101) found that the globules of 100 cc. of blood contained less copper than the corresponding plasma or serum. The quantity of copper contained in the whole blood does not seem to be affected by the ingestion of the metal in a soluble form, but in this case the globules contain more than the corresponding plasma. It seems, in general, that the proportion of circulating copper does not suffer noticeable variation from the copper content in the food ration, which is not true for the storage organs. In summarizing, Guillemet said that it was impossible to draw general conclusions from these results as to the distribution of copper in the blood and the variation of this distribution.

In another paper by Guillemet and Gosselin (102) the statement was made that crystallized hemoglobin seemed to contain much more copper than the preceding precipitations made on the globules, which leads to the hypothesis that the hemoglobin crystals carry away some of the copper with the liquor of crystallization. Attention is called to the fact that the total quantity of copper contained in the whole-blood globules of the initial blood is always more than the quantity of copper found collected in the dry hemoglobin.

In a third paper, Guillemet (103) stated that only small quantities of copper were found in dogs. A total of 0.04 mg. of copper was found in the spleen of a dog of 10 kg. bled 4 times and fed 0.1 mg. copper per day; 0.06 mg. total copper was found in the spleen of a dog bled 4 times and fed 10 mg. of copper per day. Whatever the origin of the animal, the amount of copper fed, or the bleeding of the animal, very little copper was found in the spleen. Poor nourishment, accompanied by bleeding, produced a net decrease in the amount of copper in the liver. Administration of large quantities of copper caused an accumulation of the metal in the liver.

According to Perla and Marmorston-Gottesman (104), the liver of the rat contains one-twentieth to one-thirtieth the percentage weight of copper found in the liver of the rabbit, guinea pig, sheep, or ox. This is probably a result of the great difference in the copper content of the food of these animals. The fodder of the guinea pig and the rabbit contains large amounts of green vegetables very rich in copper.

Gordon and Rabinowitch (35) referred to analyses recorded by Cherbuliez and Ansbacher of livers of 9 Korean patients without and 13 with cirrhosis. The data from these analyses are summarized as follows: The maximum, minimum, and average amount of copper, in milligrams per kilogram, of fresh material found in the livers of the 13 cirrhosis patients was, respectively, 85.6, 2.9, and 20.8; the respective amounts in the 9 patients without cirrhosis were 9.9, 0.6, and 5.4; the total milligrams of copper per organ were 117.0, 3.31, and 35.5, respectively, for the 13 patients and 21.39, 0.72, and 11.3 for the 9. It is stated that there is remarkable uniformity with respect to the average copper content of "normal" livers. They report (35) a case of yellow atrophy of the liver in which 174.2 to 185.3 mg. copper per kilogram of fresh material was found in the liver according to the method used.

In another article Rabinowitch (105) stated that copper is a universal constituent of biological material and failure to detect it in the past was due largely to the limitations of methods then available for its detection when present in minute quantities. As it appears that it is an essential element rather than a

contamination, it is to be expected that copper would be found in urine normally. The amounts found by Rabinowitch ranged from traces to 0.4 mg. per liter and from traces to 0.7 mg. per 24 hours. In two copper "balance" experiments in which the subjects were fed copper, the amounts were appreciably larger.

According to a letter to the Journal of the American Medical Association from London (106), an investigation by two chemists of the unexplained deaths of cattle grazing in different parts of the county Durham showed that the cattle had absorbed lead and copper from the grass, which had been contaminated by emanations from coke ovens in the vicinity. Stomach tissue, kidneys, and liver of 1 of the animals contained, respectively, 3.2, 2.1, and 3.5 p.p.m. of copper. The various kinds of dusts deposited in different open areas of Newcastle and on shelves of houses, porticoes, and the entrances to public buildings revealed lead to the extent of 3,000 p.p.m.; copper, 1,600 p.p.m.; zinc, 4,700 p.p.m.; and arsenic, 400 p.p.m.

Remy (107) found the mean copper content of cow, human, and goat milk to be 0.13, 0.14, and 0.09, respectively.

Rees (108) found 24 mg. of copper per kilogram in fresh ox liver. By extraction with water at 50°, 1.75 mg. of copper were obtained per kilogram of liver.

TOXICITY OF COPPER

There seems to have been, and still is for that matter, no unanimity of opinion regarding the toxicity of copper. In 1822 Patissier (109) published a translation of Ramazzini, to which he added his own observations. In addition to the information furnished by Ramazzini, Patissier related that copper miners were subject to colic, diarrhea, and dysentery. In 1877 Chéné (110) called attention to the diverse opinions found in the literature dealing with the action of copper on the human organism, some authors considering this metal a most toxic agent, others considering it harmless; as early as 1754, according to Chéné, Eller refused it all toxic action, and in 1855 Galtier concluded that metallic copper is not a poison. Even those who worked with copper disagreed as to its effects; some admitted copper colic, and others denied its occurrence. In 1883 Gautier (111) called attention to researches that had shown copper was not as toxic an agent as had been believed for centuries. Lehmann (58) mentioned that in 1889 he had called attention to the numerous unsolved contradictions in the hygienic and toxicological estimation of copper. He stated that the literature on the toxicology of copper was very voluminous and as rich in animal experiments, in clinical and forensic observations, as in critical and uncritical attempts to harmonize the results of objective research with popular opinion, old wives tales, and old prejudices rooted in antiquity and pointed out that for a century the question of the extent of the harmfulness of copper had been springing up continually and that the opponents and the supporters of copper had been irreconcilable.

As recently as 1951 Luchsinger (112) referred to the toxicity of metallic copper and its compounds as still a matter of dispute. That there is an occupational hazard from these substances is challenged, and pure copper is considered nontoxic by many authors.

In his summary of the voluminous literature on copper from the standpoint of legal chemistry, toxicology, and hygiene, Tschirch (15) concluded that, as copper cannot be entirely eliminated from food, it would be better to determine the maximum content that would be permissible. Based on experience with animals and human beings and on the experiences collected in legal medical practice, the question "Is copper a poison?" could be answered "Yes," with the qualification that it seldom causes death in human beings. However, more or less severe intoxication

can be produced when copper is taken in not too small or not too large (immediately causing vomiting) doses. "After all," according to Tschirch (15), "we cannot consider the copper compounds poisonous in the same sense as arsenic, lead, quicksilver, and phosphorus, as after administration of copper has ceased the disturbed function of the organism returns to normal."

Corrigan is quoted by Chéné as saying that the carbonate of copper acts as a slow poison, producing emaciation, catarrhal cough, gradual debility, blue lines on the gums, and loose teeth but never acute colic with constipation or localized paralysis as in lead poisoning, and that copper in chronic poisoning disturbs especially the digestive and assimilative functions. Others claimed that copper compounds might cause a rapid intoxication.

Tardieu and Roussin stated that poisoning by copper preparations was one of the most frequent, standing next to arsenic and phosphorus in criminal statistics and occurred in numerous cases accidentally or resulted from the use of copper utensils and, moreover, attacked the workmen in certain industries (113). In referring to the statements of Tardieu and Roussin, Lehmann (58) in 1897 said they were not true. Their assertion Lehmann said had never to his knowledge been proved by the production of the source materials and was evidently partly based on absolutely doubtful, superficial, and experimental poisonings only.

Chéné (110) himself carried out investigations on:

1. The action of copper sulfate in large and small doses;
2. The action of copper acetate;
3. The action of copper sulfate precipitated in the form of albuminate;
4. The action of copper sulfate administered in an aqueous and sirupy glycerine;
5. The action of ammoniacal copper sulfate in aqueous solution and as a soluble albuminate;
6. The action of copper albuminate injected into the blood.

He noted carefully the weight of the animals and the quantity of the copper preparation administered. The object was to determine the actual toxic doses capable of causing symptoms and sometimes fatalities. He found that copper sulfate in large doses could not be regarded as inoffensive, but its introduction into the stomach of animals that vomit does not cause fatal symptoms in the majority of cases. The gram per kilogram of body weight required to cause death to a dog with young is reduced to 0.25 gm. with the rabbit, an animal that does not vomit. He found that copper sulfate in small doses had no appreciable effect on the organism and, that tolerance could be established by long-continued use. He was not able to poison dogs by having them take voluntarily with their food rather large quantities of copper sulfate in powder form. Copper acetate was more toxic in large doses than the sulfate; death occurred in a large number of cases with a dose of 45 cg. for dogs and 5 cg. for rabbits, per kilogram of body weight. Insoluble copper albuminate introduced into the stomach of dogs in very large amounts did not greatly jeopardize the life of the animals. However, with rabbits, which do not vomit, the acetate was as active as the sulfate. Sulfate of copper dissolved in viscous glycerin was much more toxic than when administered in aqueous glycerin. Ammoniacal copper sulfate was more active than ordinary sulfate; soluble albuminate, obtained by combining the copper sulfate with albumin, had almost as serious

effects. Individuals who for whatever cause did not vomit or who vomited less than others were more susceptible to poisoning. An empty stomach was more favorable than a full one for rapid absorption of the poison. Soluble copper albuminate introduced into the blood killed as soon as the injected dose passed 1-1/2 mg. per kilogram of weight of dogs. Whatever the copper compound used, poisoning by stomach occurred only when the quantity of the poison absorbed was equal to the minimum toxic dose by direct injection into the blood. The principal toxic action was on the blood, the nervous or muscular system, and the digestive tract. The principal paths of elimination of copper were found to be the intestine, the liver, and the kidneys.

Feltz and Ritter (114) also observed that the less the animal vomits the more serious the symptoms caused by copper salts. Of all the copper salts ingested into the stomach, ammoniated copper sulfate caused the most energetic action. Of 3 dogs that received 0.078 gm., 0.127 gm., and 0.315 gm. of copper in the form of a 40 per 1,000 solution of ammoniated sulfate, 2 died and the other was very ill for several days. Autopsy revealed evidence of bloating, hyperemia with hemorrhagic spots, and epithelial desquamation of the mucous membrane of the stomach and intestine; analysis of the liver showed 0.004 to 0.005 gm. of copper in this organ. The signs observed during life was vomiting of serous and frothy material at first, later becoming bloody, bilious blood-tinged diarrhea, anorexia, great loss of weight, and paralysis of the sphincters. Two dogs weighing 5.1 kg. and 6.8 kg. died in 24 hours after receiving 0.6 and 0.44 gm. per kilogram, respectively, of copper in the form of soluble albuminate. The solution was administered through an esophageal catheter 2 or 3 times a day. The livers contained, respectively, 0.003 and 0.0045 gm. copper. A third dog weighing 20 kg. received only 0.15 gm. copper per kilogram of weight, but the dose was repeated during 5 days. The animal was ill from the first dose and lost 3 kg. in weight; it presented the usual signs of poisoning and on the sixth day had icteric hemorrhage. The liver contained 0.0106 gm. copper. Two dogs weighing 8.7 kg. and 13.2 kg. received in 1 day through the stomach in the form of insoluble albuminate 0.332 and 0.109 gm. copper per kilogram of weight. The animals vomited several times but did not become ill and lost neither appetite nor weight. Two other dogs of 9.7 and 17.8 kg. received 4 successive days a daily dose of 0.262 and 0.142 gm. copper per kilogram of weight without causing more symptoms than exhibited by the preceding 2 animals. Soluble albuminate of copper administered to the stomach caused as severe symptoms as ammoniated sulfate dissolved in distilled water. It was found that copper sulfate dissolved in a sirupy glycerin was much more toxic than the same salt dissolved in aqueous glycerin. A solution of albuminate of copper with titer of 0.00115 gm. copper per cubic centimeter, injected into the blood, caused death as soon as the dose passed 0.0015 gm. per kilogram of body weight of the animal. The principal portals of elimination of copper, in order of importance, seemed to be the intestine, the liver, and the kidney.

Galippe (36) gave copper salts to dogs, mixing it with their food, which he said was the only physiological process. Six gm. of tribasic copper acetate produced vomiting but never death. Small doses (50 cc.) had the same effect. He concluded that copper was not toxic and that, moreover, the disagreeable taste of copper salts would prevent their absorption by immediate warning.

Gautier (111) did not go so far as to say that copper was harmless, but he attributed to it only a very inferior toxic action. The violent emetic properties of cupric preparations caused him to recognize their toxicity. He quotes Perren and Pietra-Santa as stating, after minute observations, that the copper colic of Corrigan does not exist. However, Gautier appeared to attach little importance to copper poisoning.

Galippe (37) did not believe that canned peas containing up to 15 and 20 cg. of copper per kilogram would be toxic.

Harnack's preparation, which he called tartrate of copper oxide-sodium hydroxide, was used for experiments on the toxicity of copper by Gienandt (87). Two solutions were used diluted until a cubic centimeter contained, respectively, 0.1 and 0.006 gm. of copper oxide. Guinea pigs were used as experimental animals, and the solution was administered subcutaneously. Previous laboratory experiments had shown that about 0.02 gm. copper quickly caused fatal poisoning. This amount was usually given by Gienandt, but in some cases the dose was 0.024 to 0.03 gm. The animals died 5 to 6 hours after being given these doses, with symptoms of great weakness; 2 or 3 hours after administration of the copper the respiration was labored, occasionally audible, then it became continually slower after 4 hours until death. A certain rigidity of the hind extremities was observed after 4 hours; after 5 hours the animals remained lying still, the paralysis apparently having progressed considerably. Great restlessness was observed shortly before death, then the animals fell into convulsions and died. As the fatal dose was so small, very little copper was expected or found in the individual organs. With general copper poisoning Gienandt found copper constantly in the stomach, kidneys, and blood.

According to Tschirch (15) there is nothing to indicate that there is chronic copper poisoning. If the minimal dose is exceeded, acute symptoms of poisoning appear. He said that 50 mg. copper per kilogram canned goods could be recommended without hesitation, as this dose would not cause slight diarrhea even if an entire kilogram of canned goods were consumed daily.

Filehne (115) found that copper (in the form of double salts of sodium) was much more toxic than Harnack's findings of 0.05 CuO administered subcutaneously and 0.01 to 0.015 CuO administered intravenously to rabbits (without statement in regard to body weight). Filehne caused subacute fatal poisoning by administering 7.5 and 8 mg. CuO, in the form of double salts per kilogram of animal per day, once for 11 days and once for 13 days. With 0.003 CuO per kilogram of body weight (2 experiments) only mild diarrhea occurred (after 12, 19, 24 days). A rabbit weighing 2,150 gm. was killed by the subcutaneous application of 1 dose corresponding to 0.025 mg. CuO after 24 to 30 hours; in 2-1/2 hours it became weak in the legs. Another of the same weight died after a dose corresponding to 0.0375 mg. CuO in about the same length of time. A dose corresponding to 0.015 mg. CuO per kilogram is designated by Filehne as fatal in every case when administered subcutaneously. A dose of double salts corresponding to 0.0035 mg. CuO, introduced intravenously, killed a 1,300 gm. rabbit in 1 to 1-1/2 hours; another rabbit weighing 1,400 gm. was not affected by a dose corresponding to 0.00175 mg. CuO. Filehne considered the certainly fatal intravenous dose to be between 3 and 5 mg. CuO for the average rabbit, that is, about 2 to 4 mg. per kilogram, and the fatal internal dose to be about 15 times as large as the subcutaneous.

Filehne (115) was surprised to find that the fatal dose for potassium-copper tartrate was twice as large as for sodium salt; 0.065 mg. Cu in the form of potassium salt was borne as well as 0.03 mg. Cu in the form of sodium salt. This aroused the question whether potassium salt was less toxic in itself or was poorly resorbed. From experiments with the diffusibility of both salts, Filehne found that in the form of sodium salt of 50 mg. Cu administered, 17.45 mg. were eliminated with the urine, whereas of the 55 mg. Cu in the form of potassium salt only 15.7 appeared in the urine. The difference in time was especially striking. The entire elimination in the animal taking sodium salt occurred on the second day and was entirely completed after 60 hours, whereas, with the animal taking potassium salt, the maximum elimination occurred first at the end of 108 hours. The elimination

relationship could not explain the difference in the toxicity. An experiment was made with 2 animals of the same weight that received 0.03 Cu per kilogram, one in the form of potassium double salt and the other in the form of sodium double salt. The salts were administered to the stomach by catheter until both animals had received 60 mg. Cu in 15 ccm. H₂O. Both were killed after 36 hours. During this time more than half of the copper administered in the form of sodium double salt was eliminated in the excrement, and more than half of the copper was held in the liver. Only 4.45 mg. Cu (the deficit) had passed this barrier. With the potassium the case was entirely otherwise. The liver had only 3.18 mg., that is, only a little more than one-twentieth of the total amount and only one fourth of that found in the animal taking sodium, and the deficit was smaller; altogether scarcely half as much of the copper was resorbed by this animal as by the animal taking sodium. In the stomach and small intestine of the animal taking potassium, three times as much copper was found as in the other. In other words, in spite of its great diffusibility, the potassium-copper tartrate was resorbed more slowly and in smaller amounts than the sodium salt. This, according to Filehne, explained why the potassium salt, when taken into the stomach, was only half as toxic as the sodium salt.

As a result of experiments to determine the pathological effect of copper poisoning, great anemia was found of all the tissues and organs. The fact that the urine was clear for the first 36 hours in nonfatal cases of poisoning and then became dark in color and for several days had considerable iron content led Filehne to believe that this was the result of destruction of the blood corpuscles.

Filehne (31) gave a dog weighing 7,050 gm. enough cupratin per day to equal 2.6 gm. Cu in 20 days. The dog not only remained lively, but when it was killed the organs, especially the liver and kidneys, showed none of the changes characteristic of subchronic or chronic copper poisoning. A dog weighing twice as much and receiving in its food for 60 days 2 gm. of Cu in the form of tartrate of copper and potassium clearly showed the changes claimed for copper poisoning (the sodium compound had a still more poisonous action). The first time the animals were fed the cupratin, there was no difficulty; very soon, however, they refused to eat the food or, driven by hunger, ate only a part of it. The 7,050 gm. dog, fed in all 2.6 gm. Cu lost 1,100 gm. in the 20 days. The subchronic anemia so characteristic of copper poisoning did not develop, however. Three grams of cupratin sometimes caused vomiting; over 3 gm. given to dogs weighing about 7 kg. nearly always caused vomiting. Cats weighing 2-1/2 to 3 kg. did not vomit after receiving the first dose (3.68 percent) of cupratin (0.1012 Cu), but with larger doses, for example, 4.25 gm. cupratin with 0.1564 Cu, they vomited; but the vomiting followed much later than with the administration of the same or smaller amounts of undisguised copper. The fact that the larger doses caused vomiting and only minimal amounts of copper were absorbed from the small doses that did not cause vomiting explains the lack of toxicity of cupratin for dogs and cats with the administration of doses even up to a Cu content of 2.6 gm. in 20 days.

Although he had made no experiments on man, Filehne (31) judging from the results on animals said that Cu albumin with a Cu content of essentially under 0.05 gm. per day could exert no disturbances worth mentioning in an adult.

A dog weighing 6-1/2 kg. bore without vomiting 0.27 gm. Cu in the form of copper stearate. In this case as with cupratin the animal lost his appetite, and the doses had to be reduced. With a daily dose of 0.05 Cu in the form of stearate, which had 99.7 percent of the claimed copper content, enough food was permitted so that when after 2 months the animal was killed it weighed 6-3/10 kg., compared with 6-1/2 kg. at the beginning. The stearate was almost tasteless and odorless, and at the beginning the dogs could be fed up to 1 gm. Cu. Very soon, however, they

refused the food, even when it contained only 0.1 gm. Cu. Cats were still more wary; they seemed to be more susceptible than dogs to the acute action of stearate and after a dose containing 0.1 gm. Cu appeared to feel badly all day. Filehne found that, when fed for a long time, copper stearate was toxic. It caused the often described degeneration, especially of the liver and later, but to less degree, degeneration of the kidneys. He did not succeed in causing acute fatal poisoning in dogs or in the still more susceptible cats with copper stearate. Vomiting and the liver protected against this. When copper caused death it was not the albumin compound but the aliphatic compound of copper.

Filehne (31) carried out experiments to determine the toxicity of copper dust, such as might be breathed by coppersmiths or other workers in copper. A dog weighing 7,400 gm. was used as a subject for 2 months. On the first day he received 0.1 gm. cuprum reductum in the food he usually ate. The dog did not vomit and remained lively. On the next day he received 0.2 gm. and immediately vomited. On the third day 0.05 gm. was given in the food; he ate it without harm but vomited after 3 or 4 hours. Mostly he bore 0.025 to 0.033 gm. but at times vomited after 0.033 gm.; however, he never refused the food. The metallic copper produced vomiting with smaller doses and quicker than did the stearate. After the animal had received 2 gm. Cu in 2 months it was killed. In spite of the same length of the test period and virtually the same dosage, section and microscopic examination showed fewer lesions with the dog taking metallic copper than with the dog taking stearate. The kidneys showed virtually nothing, and the liver had suffered but little.

In 1897 in the part of the report on hygienic studies on copper dealing with its action on human beings, Lehmann (58) published a table listing cases of copper poisoning collected from the literature. He expressed doubt as to whether some of the cases quoted were really due to copper poisoning. However, he felt compelled to conclude only that although doses of 30 gm. sulfate or acetate are frequently fatal they are not always so, as poisoning by smaller known doses (3 gm. acetate and the like) has always ended in recovery. He stated that, in any case, up to that time he knew of no instance in which 250 to 500 mg. Cu - the largest dose that can be taken unnoticed with food - had caused fatal illness in a healthy adult.

Lehmann (58) quoted Toulmouche on experiments made in 1840 to test the emetic action of copper; Toulmouche gave the following amounts of copper to 75 healthy persons or persons slightly ill with grippe or stomach troubles, mostly women: 0.1 gm. (25 mg.) Cu caused vomiting 11 out of 12 times, usually 1 to 3 times; abdominal pains were experienced to some extent with watery stools infrequently; 0.2 gm. (50 mg.) Cu caused vomiting in 4 of 37 cases; usually abdominal pains were present and watery stools; 0.3-0.4 gm. (75-100 mg.) caused vomiting, with only 1 exception, in 18 administrations and watery stools; 0.4 gm. (100 mg.) Cu given to a woman once and 0.6 gm. (150 mg.) Cu given to a man once, both of whom were suffering from stomach troubles, had no effect. According to Lehmann, Toulmouche concluded his observations by saying that the pharmacology of his time was wrong in considering copper as dangerous.

Lehmann (58) also quoted the following from a statement published by Toussaint in 1857:

1. Pure copper, black copper oxide, and copper sulfide are entirely harmless for the healthy.

2. Vomiting is caused in human beings as follows:

	Gram
By sulfate of ammoniated copper oxide	0.4
By copper iodide	0.48
By copper phosphate	0.60
By copper carbonate	0.60
By copper nitrate	0.84
By copper acetate	0.84

These substances, Toussaint found, could be taken in divided doses for a much longer time without harm.

3. Food eaten at the same time, even the lactic acid-containing sauerkraut, has no effect on the action of these substances.

Lehmann (58) and coworkers experimented and found that 120 mg. Cu in the form of salts, taken with food, is harmless when the dose is divided over the day. If taken at one time vomiting may result. Many persons can take as much as 200 mg. Cu with no effects other than vomiting. He found statements by Crocq in sharp contradiction to his own experience. Crocq found that many persons could take without vomiting 10 mg. but not 30 mg. copper sulfate administered during the entire day. Lehmann said that if this observation were true it should be attributed to idiosyncrasy, to which are due so many puzzling experiences, and has no significance from the standpoint of practical hygiene as there are hundreds of harmless things, such as crabs, sauerkraut, cold milk, and strawberries that cannot be eaten by some persons.

In 1900 Lewin (22) said that no other substance had produced, with reference to its toxicological significance, so numerous contradictory opinions, experiences, and experimental results as copper; since the middle of the last century this contradiction had appeared in numerous scientific reports and that even at the time he wrote there was no agreement as to whether metallic copper or its salts could cause chronic poisoning in man. He said that this lack of uniformity was due partly to the manner in which different medical or nonmedical investigators used certain animal experiments for a decision and partly to the failure to review sharply the results. According to Lewin:

Rademacher took daily for 8 months 0.24 gm. copper oxide, or a total of 58 gm. He detected no harmful effects therefrom.

Toussaint took copper oxide morning and evening for 14 days, beginning with 0.12 gm. and increasing the dose up to 0.5 gm. With the exception of a strong copper taste, he experienced no effects. Analogous experiments were made by Burg who gave daily for a month 0.2 to 0.3 gm. copper oxide to a hundred people. Even diabetics bore the material without nutritional disturbances. Epileptics were treated with cuprum sulfuricum ammoniatum, some of whom received the substance during a half to 1 year in total amount of 43 to 124 gm. With all patients the appetite remained normal and the nutrition suffered no disturbance. Stomach pains were absent, although finally transitory abdominal pains were experienced by almost all and in only four were vomiting and diarrhea observed. One epileptic was given 10.2 gm. cuprum sulfuricum ammoniatum in 39 days, beginning with 0.12 gm. daily in two doses and after 11 days 0.72 gm. daily. Then the doses were reduced. During the entire time vomiting occurred only on 2 days. Copper taste was often present, as was to be expected. The urine always contained copper. Another epileptic received

in 36 days a total of 17.3 gm. of cuprum sulfuricum ammoniatum and only on 1 day did vomiting occur. Otherwise there were no toxic symptoms.

A sick person used in 25 days 18.4 gm. copper nitrate. After 14 days he was taking 1.56 gm. daily without any disturbance. On further use on some days vomiting and abdominal pains occurred. Copper carbonate, of which a sick person took 7 gm. in 10 days, caused abdominal pains and vomiting only once, while with the use of 7 gm. of cuprous sulfide not once did these symptoms appear in 7 days, not even a copper taste, and copper was not present in the urine.

A patient received in 21 days 16.3 gm. neutral, that is, basic copper acetate, beginning with 0.3 gm. and after 8 days he took morning and evening 0.78 gm., also on one day 1.56 gm. without any particular symptoms. When on the following day 0.84 gm. was taken vomiting resulted. This did not recur although after reduction of the dose for one day to 0.6 gm. the dose was again increased to 0.84 gm.

Toussaint himself took a copper preparation daily for 6 months and remained very well; Meyerhardt took daily for 50 days 0.0393 gm. copper sulfate and for 30 days 0.0786 gm. of this salt, or in 80 days 4.323 gm., with the same negative results.

Lewin himself swallowed 1 to 15 gm. of very finely divided metallic copper without the least difficulty, although vegetable acids were taken afterward. In a series of experiments in which 12 dogs were used, finely pulverized copper up to 30 gm. was given without injury. On the day after, much copper was found in the excrement, and the animals also showed no symptoms of poisoning when, after administration of 15 gm. copper filings, much fat or oil or vinegar was introduced into the stomach. In other experiments a dog was given with its food for 2 months a total of 2 gm. of finest metallic copper without the appearance of symptoms except occasional vomiting. The changes found in the kidneys (2 to 4 small globules colored dark brown by osmium and a few granules) when the animal was killed were not considered by Lewin as due to disease.

Concerning the toxicity of copper and lead, Lewin (22) quotes the following statement by Bouchard as fundamental: "From the point of view of hygiene lead has caused more harm than fear and copper more fear than harm." In this way, according to Lewin, most of those descriptions of supposed acute poisoning by copper or its salts and their aftereffects, which have filled toxicological literature for almost two centuries, will be placed in the correct light. A considerable number of these descriptions assume copper as definitely the source of poisoning where this metal served accidentally as the carrier of organic substances that had become toxic through decomposition. To this class, for example, belong those cases in which wounds were caused by dirty copper and where the organic infections caused lymphangitis and its results or those in which frightful suffering was reported to have occurred from taking food and drink that had been cooked in copper vessels not at all or imperfectly tinned or had remained therein for some time uncooked. Also here, says Lewin, the true cause of sickness could be referred with probability bordering on the truth to the processes of decomposition within the food concerned.

In fact, according to Lewin, many of the older authors themselves who hold entirely to the view that food prepared in copper vessels can be poisonous and communicate the correspondingly supposed poisoning, it appeared finally to be advisable to limit the generality of the statement. At the beginning of the last century such an author wrote:

As long as the surface of the cooking vessel is kept clean, dry, shining, and free of all rust, as long as it is kept in a place where it has nothing to suffer from sharp corroding vapors, as long as one does not allow acids, fats, wine, milk, beer, tea, syrup, etc., as long as nothing is cooked in it which has in it an evident sharpness, acid, or lye salt combined with it, or if one should cook something of the sort therein as soon as it is cooked it is emptied out and not allowed to cool therein, as long as nothing is kept therein continually, these dangers are sufficiently removed.

"It is seen," said Lewin, "that these limitations essentially agree with the ones made in our time when it is desired to reject the assertion of the general harmfulness of the use of copper vessels for cooking."

Lewin stated, however, that there is one case in which without doubt copper is the cause of poisoning and that is the acute poisoning following the ingestion of large quantities of soluble copper salts with suicidal intent, because with the involuntary ingestion of copper compounds the peculiar metallic taste serves as a warning of the danger.

Titze and Wedemann (116) administered 71 gm. copper sulfate to goats in 61 days; after an interval of 50 days one of the goats received 82.5 gm. copper sulfate in 61 days and the other goat received 60.5 gm. copper sulfate in aqueous solution in 50 days. Copper was not eliminated in the milk in either period. No effect was observed on the amount and composition of the milk from the feeding of the copper salts. Disturbances to health or changes in organs, such as changes in the cellular constituents of the blood, did not appear as a result of the administration of copper salts. By far the greater amount of copper was found in the liver, next in the kidneys, and then in the blood. The udder, muscles, and fatty tissues were free of copper. The results of these investigations agree with statements in the literature.

Harnack (23) said in 1914 that, in spite of numerous searching investigations in the field of copper poisoning, an apparent contradiction still existed, and the toxicity of copper had been at various times very differently evaluated. These apparent contradictions were due to the fact that either the conditions were seldom right for the passage of toxic amounts of copper into the blood, there was something in the chemical composition of the copper which reached the blood, or, there was some significance in the place of application; there were more dangerous places of application of heavy metals than the stomach. He called attention to the fact that 40 years before he had pointed out that copper was one of the most intensive muscle-paralyzing poisons, with paralysis of the heart muscle causing death. When copper entered directly into the blood in certain combinations, it could exert the most dangerous effect and as a poison was about on a level with arsenic.

Finding copper in greatest concentrations in some of the most vital organs of plants and animals led McHargue (60) to infer that this element had important biological functions in the normal metabolism of plants and animals.

Thompson (25) expressed the opinion that probably many of the symptoms attributed to copper were due to associated metals with which it is found.

According to Preble (26), when swallowed in considerable quantities soluble copper salts are not only acutely irritant but are systemic poisons like salts of mercury or lead. In large enough quantities copper salts set up an acute gastroenteritis, which is severe enough to cause a certain amount of collapse, but whether this action alone is sufficient to cause death, as is the case with other

violent irritant poisons, is difficult to determine because the entrance of the mineral into the blood causes another train of symptoms; these consist of alterations in the blood, kidneys, liver, and heart. Thus hemolysis is encountered with extravasation of blood, nephritis - or in modern parlance a toxic nephrosis - with anuria, fatty degeneration of the liver, and fatty heart. Preble stated that copper poisoning in mild form is very common because copper salts are widely used and easily obtainable; although sometimes taken with suicidal intent or for simulation of disease, they are not infrequently swallowed by mistake and in some parts of the world are used like lead salts in large quantities to produce abortion; however, the known and authenticated fatalities are few. He found gastroenteritis to be an occasional sequela. Prognosis should be good as very large amounts of the salts had been taken with relative impunity. He recommended for treatment washing out the stomach; if at a distance from medical aid, white of egg and milk could be used as first-aid antidotes; potassium ferrocyanide is the chemical antidote and may be added to the lavage water; collapse should be treated in the usual manner by warmth, heart stimulants, and similar measures.

The systemic toxicity (tested in mice) of copper was found by Corper (117) to be about the same for copper sulfate and the copper amino-acid mixtures based on equal copper content, the lethal dose being between 0.05 and 0.1 mg. copper administered intraperitoneally for a 10-gm. mouse. When given intramuscularly, absorption was uncertain, owing to the protein combinations formed by copper in the tissues, and comparable results were not easily obtained, but the approximate lethal dose was 0.1 mg. copper for a 10-gm. mouse. Absorption of copper by inunction in guinea pigs was tried, but the results were unsatisfactory. Copper is absorbed by inunction after administration of copper oleate in lanolin or copper amino-acid solutions painted on and allowed to dry on the skin. A guinea pig that received 2 gm. of 50-percent copper oleate (0.1 gm. copper) and then 4 days later received 2 gm., 8 days later 3 gm., and 7 days later 3 gm. was dead on the ninth day after the last inunction. The total liver, 11 gm., yielded 0.3 mg. of copper.

The acute and chronic toxic properties of 4 copper salts - 1 inorganic preparation, copper sulfate, and 3 organic preparations, copper glycinate, copper glutamate, and copper leucinate - were investigated by Huber (118) with the following results:

An attempt was made to produce chronic intoxication by subcutaneous injections, intramuscular injections, and feeding. Corper's method of making copper determinations, with slight modification, was used.

Copper, whether in the form of sulfate, leucinate, glycinate, or glutimate, shows no variation in action when introduced into the conjunctiva of rabbits. The lower dilutions, 1 percent, produce hyperemia and lacrimation to the same degree, while the higher dilutions, 0.1, 0.05, and 0.01 percent, were inactive. Copper in the salt forms named showed very little if any variation in ability to produce acute intoxication when introduced in dilute solutions subcutaneously. The dilution was important as the local injury produced by the higher concentrations interfered with absorption. The toxic subcutaneous dose of all the salts was found to be between 4 and 6 mg. per kilo for guinea pigs.

Copper in the forms used showed no variation in action when introduced intracutaneously. The lower dilutions caused necrosis and induration and the higher dilutions caused little or no change. The histological picture with the same dilution of all salts was the same.

These forms of copper showed no variation in action when introduced in small gradually increasing doses, 0.5 to 1 mg. copper per kilo, for a long period of time, 105 days, either subcutaneously or intramuscularly. This dosage of copper seemed to produce no gross or microscopic changes in the liver, kidney, spleen, heart, or lungs, although the liver and kidney both showed marked increase in amounts of copper when analyzed.

Copper in the salt forms named showed no variation in ability to dialyze through celloidin sacs when either water or horse-serum was used both inside and outside the sac; the rate of dialysis was somewhat faster with distilled water than with horse serum. It showed no variation in action when introduced by feeding in small gradually increasing doses, to 10 mg. per kilo per day, for a long time, 72 days.

The deposition of copper in the liver and kidney and the lack of microscopic changes in the tissues examined, simulate the findings of the subcutaneous and intramuscular experiments with copper. The three copper amino acids examined seemed to produce exactly the same physiological effects as a simple inorganic salt, copper sulfate.

Drummond (40) undertook experiments to clear up two questions: Is copper liberated in assimilable form during digestion of greened vegetables, and, if so, does the absorption of copper in small amounts over long periods cause damage to the organism? Experiments were made in vitro and in vivo. In the experiments in vitro, the amount of copper to pass through the dialyzed membrane in a certain time was very small. In the digestion of 100 gm. of timed peas calculated to contain about 5.6 mg. copper, only 0.01 to 0.02 mg. was detected in the outer fluid after 2 hours' digestion. The total amount passed out in 24 hours was 0.05 mg. or 0.9 percent. Uncolored peas used as controls also contained small amounts of copper, but the amount passed out from the digestions was too small to estimate. The experiments in vivo confirmed in all essentials those in vitro. A very minute amount of copper was absorbed from the normal peas, whereas from the "copper" peas the total amount appeared to be of the same order as in the test-tube experiments, rather less than 1 percent.

According to Drummond (40) an extensive series of experiments by Long and Chittenden failed to establish that the consumption of "greened vegetables" is deleterious. He also called attention to the warning of Mallory, Parker, and Nye, who claimed that prolonged administration of copper salts to certain animals caused hemachromatosis and stated that their experiments lost value because they gave much larger doses of copper salts than are ever likely to be taken in the form of colored vegetables and also because hemachromatosis is not associated with workers exposed to copper poisoning.

Drummond (40) made experiments on rats in which a 100-gm. rat ingested approximately 1.5 mg. copper daily; throughout the experiments, which lasted 3 months, the animals showed good health and normal behavior. Definite evidence was found that animals can absorb traces of copper, and part of this metal is held for a time by the liver until it is excreted either by way of the urine or the feces. However, his experiments with rats made proof that absorption of traces of copper over long periods of time is likely to lead to harmful consequences questionable.

According to von Potheo (119):

Cases of copper poisoning are rather unusual; when they occur now and then the course is usually light and conceals no serious danger in itself, since the immediate vomiting protects the organism from resorption of larger amounts of copper and usually results in only slight colic and diarrhea. When, however, severe poisoning is accomplished by mouth, principally stomach and intestinal symptoms appear, later accompanied by copper neuritis and followed by death with symptoms of paralysis of the heart. Poisoning mostly occurs from eating sprayed fruit, through the use of copper vessels, and very seldom as a result of taking large amounts of copper sulfate with suicidal intent. Injection

of copper sulfate in tuberculous therapy has recently been experimented with whereby already much poisoning has arisen, even death after intravenous administration.

The following case observed in the children's clinic stands alone in the literature on account of its peculiar circumstances. A 2-1/2-year old boy was brought to the hospital with the statement that he had been suffering for 2 days with continual vomiting, evicting bloody mucus, was very feeble and restless with slight convulsions, the extremities trembling greatly and painful when touched. The child had suffered for 1-1/2 years with eczema of the head. At the suggestion of a layman, his head had been rubbed 2 days before with copper sulfate dissolved in cream. He died the next day after being brought to the hospital. By section and chemical examination diagnosis of copper poisoning could be made with certainty. The copper found its way through the wounds in the head directly into the blood rather rapidly, since the organism was in no condition to offer protection. Probably if the same amount of copper had been administered to the child by mouth he would not have died, thanks to the vomiting and diarrhea that would have resulted.

McHargue (65) stated that pigeons confined to a diet of polished rice soon develop polyneuritis, whereas pigeons fed on unpolished rice maintain a normal condition. Authorities on the subject of vitamins assume that these important factors are synthesized in plants and not in animals. According to McHargue's results, plants absorb small amounts of the elements copper, iron, manganese, and zinc from the soil and synthesize from them compounds that are not poisonous but apparently are vital factors in plant and animal metabolism. He concluded that if this assumption is correct, then copper, manganese, and zinc perform more important functions in agriculture than is generally recognized.

Mallory (32) fed fine copper powder on food to rabbits, which was dissolved in the gastrointestinal tract and caused death in 2 months, with marked pigmentation of the liver. Injected intravenously, intratracheally, or subcutaneously the copper was quickly dissolved by the fluids of the body, causing marked local reaction and deposit of pigment in the liver, kidneys, bone marrow, and some of the other organs and in some instances death in 30 to 72 hours. Chronic poisoning with copper in sheep and acute poisoning in rabbits caused, in addition to pigmentation, blocking of the tubules of the kidneys with hemoglobin casts, indicating destruction of red blood corpuscles. Hemofuscin, one of the two pigments always present in hemochromatosis, is apparently nothing but an intermediate product between hemoglobin and hemosiderin. It is deposited in the liver and other organs of animals as the result of poisoning both with copper and with zinc, but it is also found in the liver and kidney following intravenous injection of hemoglobin and in the cells of the tissues surrounding old hemorrhages. The change of hemofuscin to hemosiderin can be followed in the livers of animals poisoned with copper and also in the islands of regenerated cells in the liver of man. Microscopic examination of the liver, pancreas, kidneys, and heart in all adult human beings (463) coming to autopsy during 2-1/2 years showed hemofuscin and hemosiderin present in 54, or 11.6 percent.

From experimental work with animals, Mallory found that copper inhaled or ingested is dangerous to life, although its action as a chronic poison is exceedingly slow. This is the reason its deleterious effect has been so long overlooked. Studies of clinical cases show that ordinarily it takes 15 to 25 or more years to produce the symptom complex of the disease known as hemochromatosis.

An editorial in the Boston Medical and Surgical Journal (45) states that the presence of copper in so many substances taken into the body is rather difficult to reconcile with the rarity of hemochromatosis until it is remembered that this is a chronic disease and that the clinical symptoms do not appear until near the end. Reference is made to the finding by Mallory of hemofuscin, the characteristic pigment of hemochromatosis, in 11.6 percent of 463 autopsies. In other words, these

individuals had ingested enough copper to produce various degrees of pigment deposit, though not enough pigment had been formed to embarrass the organs and cause symptoms to appear. Production of enough pathological change to give rise to clinical symptoms, according to the editorial, probably takes years.

There is no doubt, according to this editorial (45), that the human organism can take care of minute amounts of copper. Where the dividing line between the harmless and the harmful amount comes cannot be said as yet, and individual susceptibility probably enters in to complicate the problem. From what is known of the solubility of copper and the amount of it present in various substances, it is considered fairly safe to say that the danger from copper and brass pipes is negligible, that copper cooking utensils might possibly be dangerous, especially when used for fruits, but that the real risk comes from industrial hazards and from moonshine liquor.

Sternberg (120) in 1926 warned of the danger of chronic poisoning from cooking vessels when the tin coating over copper wears off or copper vessels are used. He recommended lavage and white of egg for treatment of acute poisoning by copper salts; and finely pulverized iron or iron compounds for the chronic form, as the iron attracts the copper out of its combinations.

Tammann and Rienacker (121) found that toxic action was exerted by pieces of purified metal 6 to 8 mm. long and wide introduced into Petri dishes containing bacterial cultures in nutrient mediums (*B. coli communis* in gelatin at 20° C. and *B. brassicae*, *Sarcina agilia*, *B. gossypii*, or *Penicillium glaucum* in agar-agar medium at 35°). The strongest toxicity was exerted by mercury, copper (except on *B. coli communis*), nickel, cobalt, and antimony.

Flinn and Inouye, in an article published in 1929 (122), stated that copper is so universally distributed throughout the plant and animal kingdoms as to raise the question whether it may not play some important role in the metabolic reaction of the organism. Certainly, they said, its presence in plants and animals alike cannot be explained simply by its adventitious occurrence in the soil; there is increasing evidence that, up to a certain point, copper may be beneficial to both animals and plants, but more than this quantity sometimes, but not always, causes ill effects. They considered this particularly suggestive in view of the fact that with more accurate methods for the determination of copper it had been found that the normal quantity of the metal present in plants and vegetables was actually less than reported in the literature. The findings of Flinn and Inouye led them to suspect that the amount of copper present bears a definite ratio not to the quantity of copper present in the soil but to some compound in the plant. They observed that the amount of protein present affected the toxicity of copper, which fact was previously observed by Voegtlin, and it has been shown by Inouye that mucin has an affinity for heavy metals, which led him to believe that the mucin in man's body is one of his greatest protections against metallic poisoning. Copper salts injected subcutaneously into animals were found to combine with the proteins at the point of injection and only a very small percentage of the injected copper was recovered in the excreta and body organs. The mass at the point of injection became necrotic and later sloughed off. Only a small amount of copper was absorbed by the blood. Bacteria in water were destroyed if the water flowed over copper, but if milk was passed over copper in the same way the bacterial count was only slightly affected. This protective action of protein was evident in the feeding experiments carried out by Flinn and Inouye. If food was present in the stomach when copper was fed, the copper tended to unite with it, leaving the mucosa of the gastrointestinal tract unaffected; the copper combined with the food dissolved slowly, the rate depending on the pH of the stomach or of the intestinal contents.

Flinn and Inouye (122) were led to study some of the physiological effects of copper because of Mallory's suggestion that hemochromatosis might result from the

continuous ingestion of small amounts of this metal. In a private communication to them, Mallory affirmed that, to produce hemochromatosis, it is necessary to feed the animal more copper than it can assimilate and the excess copper causes hemolysis, with a subsequent deposition of the blood pigment in the liver, and he believed that anything causing hemolysis will produce such lesions. If Mallory is correct in his belief that copper is a hemolytic agent if taken per os and, therefore, the causative agent of such lesions, Flinn and Inouye find it difficult to explain why hemochromatosis is not found among lead workers, among whom anemia occurs as a result of the fragility of the red cells from exposure to lead. They found no evidence in their work that copper ingested by the body in the normal way acts as a hemolytic agent and were unable to find an increase in the storage of copper in the liver in any of their experimental animals, except in cases where the animals had been fed far larger amounts of copper per kilo of body weight than man would normally be exposed to, even temporarily, in his daily or industrial life. A spectrophotometric examination of the blood did not reveal the presence of methemoglobin. Also, the experiments of Flinn and Inouye did not confirm statements in the literature that copper is deposited in the bones, except in traces. Bone exposed to a solution containing 1 mg. of copper in the form of chloride formed a precipitate almost from the beginning of the experiment, a large portion of it settling around the bone, thus giving the impression that the copper salt was combining with the bone. When the bone was washed, however, this proved not to be the case. Analysis did not show replacement of the calcium in the bone by the metal. In the opinion of the authors a precipitate is formed because of change in the pH of the solution. The results of the experiments indicate that copper may play some important role in stimulating blood formation and that its constant presence in the liver, even in the liver of the fetus, may not be due merely to the fact that that organ has among its functions that of being a filter. What the combination is in which it is held could not be told at this stage of the work, but it certainly must exert an important influence on the hematopoietic system and on the metabolism of the body as a whole. An examination of the blood of guinea pigs and dogs showed that the oxygen-carrying capacity of the blood was increased when copper was fed to animals, which they thought indicated that perhaps the copper is present in the blood as a catalytic agent and may be poisoned or rendered inactive in the same way that CuCl is nullified in industrial plants by SO_3 , SO_2 , or arsenic acid.

In 1929 Polson (123) carried out experiments, which he said were a fair repetition of those of Mallory, Parker, and Nye in which they claimed to have produced an accumulation of iron-free pigment in the liver and cirrhosis of the organ following the administration of copper acetate. Polson stated that:

In 1925 Mallory claimed that hemochromatosis was a result of chronic copper poisoning and in support of this contention quoted his previous experiments. Hall and Butt in 1928 repeated Mallory, Parker, and Nye's experiments and obtained results in agreement, both in respect to pigmentation and to cirrhosis of the liver. None of these investigators made reference to normal controls. Flinn and VonGlahn, repeating Mallory's experiments in 1929, failed to produce cirrhosis but observed the accumulation of "hemofuscin" in the liver; however, they found the same result when rabbits were fed with sodium acetate, and more significantly they found that a diet of carrots caused the heaviest pigmentation of the liver in a much shorter time than did copper or sodium acetate; they also found the same result, but less severe, from feeding turnips.

Polson used the same range of dosage as Mallory, Parker, and Nye, and the experiments lasted for a longer time. Copper acetate failed to produce a pigment

cirrhosis of the liver, whereas a diet containing mangel-wurzels and turnips caused accumulation of "hemofuscin" in the livers of 87.5 percent of the controls. Polson, therefore, concluded that the diet was responsible for the striking incidence of liver pigmentation in the Leeds series of rabbits and in the failure to show pigmentation in the Manchester controls as well as in the majority of the experimental rabbits used at Manchester. He considered this to be the probable explanation of the difference between his results and those of the other three groups of observers.

Flinn and VonGlahn (97) found the results of earlier investigators to be conflicting. They used guinea pigs, white rats, and rabbits as experimental animals. The guinea pigs were fed daily for the first 5 months the copper chloride equivalent of 0.2 mg. copper and for the next 13 months 1 mg. of metallic copper. Six rats were given water containing the equivalent of 1 mg. of copper in each 10 cc. (it was found that rats of the size used drank an average of 10 cc. of water daily), and 5 rats received 2 mg. in each 10 cc. The rabbits were divided into eight groups. Some were given metallic copper in amounts varying from 20 to 320 mg. daily for 15 to 300 days; some were given 100 mg. copper acetate for 19 to 69 days; and some were given 100 mg. copper tartrate for 94 to 177 days.

The results obtained by Flinn and VonGlahn with guinea pigs were entirely negative. No pigment had been deposited in the liver and no cirrhosis had been produced. This finding is in accord with that of Huber. Mallory, Parker, and Nye found guinea pigs more resistant to the effects of copper than rabbits. The same results were obtained with white rats. Flinn and Von Glahn's findings on rats coincide with those of Drummond but not with those of Mallory and his coworkers or Hall and Butt. The following results were obtained by Flinn and VonGlahn on rabbits:

The results on the various doses of copper or its compounds were uniform for all groups of rabbits. Pigment occurred in the liver in fine refractile particles having a yellow or yellowish-brown color. There was no fixed relation between the amount of pigment and the length of time the copper was administered. No difference could be detected as regards the pigment deposition and the form in which the copper was given. No correlation could be found between the amount of copper contained in the liver and the quantity of pigment deposited. It seemed of interest to determine whether the acid radicles in the copper salts were of importance in influencing the deposition of the pigment. With this in view 10 rabbits were given 100 mg. of sodium acetate daily; at the end of 5 months 2 of the rabbits were killed and the remaining 8 were allowed to live a month longer. The findings were similar to those in the animals receiving copper. This experiment made it obvious that copper was not the agent causing the pigment deposition in the various groups of rabbits to which it had been given. From experiments with the effect of diet, it was found that when rabbits were fed on a diet of carrots exclusively, the deposition of pigment occurred with greater rapidity than with copper or its compounds and the standard diet. The changes resulting in the livers of the carrot-fed animals were identical in every way with those seen following large doses of copper and in animals given comparable doses of sodium acetate. A definite cirrhosis could not be proved in any of the rabbits given copper.

It was concluded that copper or the compounds used do not cause the deposition of pigment in the livers of rabbits, guinea pigs, or rats; neither does it produce

a cirrhosis in these animals; spontaneous deposition of pigment occurs frequently in the livers of normal rabbits on the usual laboratory diet; the feeding of a diet of carrots exclusively will produce pigment deposition in the livers of rabbits in every way identical with that ascribed to copper; the pigment deposited in the livers of rabbits is probably of exogenous origin.

Because of the identification of copper as an active agent in hemoglobin building in the rat by Hart, Steenbock, and associates, later confirmed by McHargue, Healy, and Hill, it seemed desirable to Lindow, Peterson, and Steenbock (124) to study the metabolism of this element more extensively than had hitherto been done, as observations made by several investigators on the results of feeding copper in large doses to different species of animals over long periods differed greatly as to the toxicity of this substance. Huber found that copper salts of amino acids given to guinea pigs by various methods - subcutaneously, intramuscularly, and by mouth - produced only negative results. Drummond, in studying the deleterious effects of vegetables colored with copper, and Flinn and VonGlahn, investigating the poisonous effect of metallic copper and the copper salts on white rats and guinea pigs, obtained no toxic effects; that is, they found neither pigment deposition nor cirrhosis in the liver. Mallory and coworkers found pigment deposited in the liver of the rabbit, rat, guinea pig, and monkey when large amounts of copper were fed and believed that there is a direct relation between pigment cirrhosis and copper poisoning; they consider that the liver of a rabbit suffering from chronic copper poisoning undergoes changes comparable in many ways with modifications in the human liver due to a chronic disease known as hemachromatosis.

The data presented by Lindow, Peterson, and Steenbock (124) deal with the metabolism of copper in the animal organism; more specifically they deal with the assimilation, storage, transmission to the offspring, and excretion of copper by the rat. These authors summarize their findings as follows:

The absolute amount of copper in the body of a rat on an adequate ration gradually increased from 0.0108 mg. at birth to 0.4422 mg. at 210 to 240 days. On a percentage basis there was a constant decrease from birth up to 85 days and a slight increase at 210 to 240 days. The copper content of a rat at birth was not raised by subjecting the mother to a high copper intake. There was no indication of increased placental transmission, nor was there increased transmission of copper through the milk. Feeding of additional copper led to marked increases in the absolute copper content and the percentage of this element present in the bodies of rats of various ages fed different rations. This increase was most striking in the more adult animals which at 75 to 85 days of age contained 2 and at 210 and 240 days 3 times as much copper as the animals fed on the stock ration alone. The percentage figure increased continuously after 25 days.

The distribution of copper in the tissues of two groups of adult rats, one fed the stock ration, the other the stock ration supplemented with copper, was determined. Copper feeding increased the copper content of the skeleton, the kidney, the spleen, and the liver, 1.6, 2, 5, and 20 times respectively. The copper content of the blood from adult rats fed the stock ration was 0.0543, while that from the copper-fed animals was 0.0821 mg. per 100 cc. The copper content of rats on an anemia-producing diet was the same as 3 weeks and at the time of death (9 to 11 weeks). Although the young from copper-fed females have no increased resistance to anemia at birth, they can acquire some resistance during the period between 12 and 29 days through ingestion of the mother's ration. Adult rats fed the stock

ration excreted 2 parts of copper in the feces to 1 part in the urine. When the stock ration was supplemented with copper, about 98 percent of this element was excreted in the feces. The urine copper increased to about 5 times the quantity that was present in the pre-copper period. There was a storage of copper during the feeding period. This stored copper was eliminated during 4 to 5 weeks of post-period feeding.

Santesson (125) called attention to an earlier work in which he described experiments with subcutaneous injection of rabbits with CuS and CuSO_4 , which showed principally liver changes - hemorrhages and necroses. When death in the animals was acute the destruction was very great. If the animals were killed some time (10 to 22 days) after the end of the injection period, the necroses were few and no growth of cirrhotic connective tissue was to be seen instead of necroses. Another investigation was made to determine whether a cirrhosis would appear with profoundly poisoned animals that had lived a long time afterward, with the following results:

Two animals were treated exactly alike. Beginning with one very small dose (about 2.4 mg. CuSO_4 , water-free salt, per kilo) 62 mg. CuSO_4 per kilo were injected in 8 increasing doses during 24 days. One animal died on the day of the last injection and showed extensive hemorrhages and necrotic foci in the liver. The other animal was killed 58 days after the end of the injection period. Its liver, normal on the whole, still showed individual necrotic foci but no cirrhosis. A second pair of rabbits was treated in the same manner, one animal, which had in the beginning received subcutaneously in 7 doses 44.9 mg. CuSO_4 per kilo, remained apparently healthy and when killed 63 days after the last injection showed in the liver only individual very small hemorrhages or necroses, no cirrhosis; the epithelium in individual sections was still partly degenerated. The control animal had to be killed earlier on account of an accident (the total dose was 37.8 mg. CuSO_4 per kilo) showed extensive necrosis of the liver epithelium without greater foci of degeneration. The experiments support the idea that copper poisoning in the form produced here causes no cirrhosis of the liver. The liver appears to be able to heal in an unexpected manner through the formation of new epithelial cells. Many times animals have been killed suddenly by a rather small dose (about 7 mg. CuSO_4 per kilo). If, however, one begins with a smaller dose, considerably larger doses (14 mg. per kilo) can be injected at one time without killing the animal. A sort of tolerance appears to develop.

The toxicity of metals was determined by Bertrand and Serbescu (126) by dissolving the salts thereof in a concentration of 1 gm. per liter of redistilled water and injecting the constant dose of 100 mg. per kilogram subcutaneously into guinea pigs and rabbits. The average survival time, in hours, for guinea pigs was as follows: $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.52; $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$, 1; $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$, 1.19; $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 2.17; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 3.01; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 7.14; FeCl_3 , 7.51; $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$, 8.34; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 8.44. For rabbits the corresponding average survival period, in hours, was 0.53 for copper, 1.11 for nickel, and 8.09 for aluminum.

According to Gebhardt and Sommer (127), although in the past copper has been considered toxic or undesirable in foods, today it is generally known that this metal is an essential constituent of most plants and animals and plays an important role in life processes, as has been shown definitely by the recent work of Hart and associates on the function of minute amounts of copper in foods in the

cure of nutritional anemia. Gebhardt and Sommer stated that this work opened up an important field for further studies on the functions of this element in life processes and its distribution in living material. However, in the manufacture of many foodstuffs it is still desirable to keep the copper content at a minimum for technical if not for legal reasons; this applies especially in the case of dairy products, where the detrimental effect of copper has long been known but not fully appreciated until the recent (unpublished) work of Guthrie.

In regard to the toxicity of copper to animals, Cunningham (66) stated that it is now fairly generally agreed by writers on toxicology that acute poisoning by copper, resulting in death, is rare, since toxic doses are so large that they are immediately rejected by the stomach. The position in respect to chronic copper poisoning is not so clear. He quotes Pecholier and St. Pierre as saying in 1856 that slow absorption of copper was advantageous, whereas Mallory in 1925 expressed the view that continued exposure to copper, such as is experienced by workers in copper foundries, will cause hematochromatosis; in support of Mallory, Funk and St. Clair in 1930 reported a case of hematochromatosis in man where the proportion of copper in the liver was extremely high.

According to Mallory and Parker (128), owing in part at least to suggestions made earlier by them that hemochromatosis might be due to chronic poisoning with copper, much chemical work was done more recently by Schonheimer and his coworkers on the question of an increase above normal in the amount of copper in the liver in pigment cirrhosis. In 1928 Schonheimer and Oshima reported examination of livers from 17 cases of hemochromatosis and stated that they had demonstrated an average increase in the amount of copper of 3 to 4 times the normal. Herkel, continuing their work, reported the examination of 24 additional cases. He found up to 10 times the normal amount of copper. However, he examined also 10 cases of nonpigmented cirrhosis. Two contained normal amounts of copper, but the other eight showed a marked increase, in part exceeding that present in pigment cirrhosis. As a result of his chemical examinations Mallory and Parker stated that Herkel did not feel that he was justified in holding copper responsible for causing hemochromatosis.

Using 2 very delicate chemical tests, Mallory and Parker (128) were able to demonstrate copper in the islands of regeneration in 5 cases of active pigment cirrhosis. It was found in the pigment granules of the young regenerating liver cells and also in the masses of inspissated bile occasionally present as the result of focal bile stasis. The study of the lesions in these five cases demonstrated clearly that copper is deposited in the young liver cells and is bound up with a derivative of hemoglobin with which it forms yellow pigment granules (copper hemofuscin). In the course of weeks to months the copper disappears, apparently in the bile in which it is regularly present in demonstrable quantity, and leaves behind pigment granules, which for a time may give no reaction for iron and are called hemofuscin. Both types of hemofuscin granules stain deeply with basic aniline dyes. Later these pigment granules undergo a chemical change, as a result of which they react to iron (hemosiderin). Sometimes this change takes place rather quickly while more or less copper is still present in the granules. Quick elimination of the copper is supported by work on acute copper poisoning in rabbits and other animals in which it was found that the copper had virtually disappeared from the liver within 5 months after subcutaneous administration had ceased. From the evidence presented, it was concluded that so much more iron than copper is present in the liver in hemochromatosis because the copper is quickly and steadily eliminated in the bile. The iron in the hemoglobin derivatives remains behind and accumulates for years. Apparently it cannot be eliminated until hemofuscin breaks down to hemosiderin. Then it very slowly dissolves and disappears. The injurious action of the copper is in part due to its combining with hemoglobin and causing it

to be deposited as copper hemofuscin in the liver and other organs. The cause of necrosis of the liver cells, which finally leads to the formation of pigment cirrhosis, has always been a puzzle. An equal amount of pigment in the liver cells in pernicious anemia produces no such effect. The demonstration that copper occurs focally in considerable amount in the liver in pigment cirrhosis reasonably suggests (122) that it is the cause of the lesion.

According to Ebeling (129) 0.5 mg. per liter of copper sulfate caused serious injury or death to fish. As little as 0.5 part per million of copper sulfate was definitely toxic to growing trout. The heavy metals also cause injury to fish by the flocculation action in water.

A study of the influence of Fe, Zn, and Cu salts on the growth of cultures of *Chlorella vulgaris*, *Coccomyxa simplex*, *Scenedesmus bijugatus*, and *Stichococcus mirabilis*, carried out by Roberg (130), revealed that concentrations of Zn up to 0.001 mg. stimulate, while 0.05 mg. per 100 cc. is toxic. Cu stimulates very slightly in minute amounts but is toxic in higher concentrations. Toxicity is associated particularly with autotrophic conditions, and stimulation with heterotrophic and mixotrophic conditions.

Fourteen copper salts of organic acids were compared experimentally by Janke and Beran (131) with 10 corresponding free acids and inorganic copper salts (CuSO_4 , CuCl_2) as well as with an organic bismuth and cadmium compound. With reference to their effect in preventing or restricting the development of bacteria (*Micrococcus* - *Staphylococcus* - *albus*, *Bacterium prodigiosum*, *Bacillus subtilis*) and their destructive effect on the same, as well as fungus (*Trichothecium roseum*, *Monilia cinerea*), it was found that, in general, the entrance of copper into the molecule of organic acids decreased their disinfective action. Among the copper salts, Cu salicylate gave the best results. None of the copper salts used completely destroyed the *Bacillus subtilis*.

Gordon and Rabinowitch (35) stated in an article published in 1933 that the data presented by Mallory and his coworkers in regard to chronic copper poisoning causing hemochromatosis was not convincing. In this connection the former called attention to the statement of Mills that Koreans, who are continually exposed to copper, show no disturbance of pigmentation, and diabetes, a condition commonly associated with hemochromatosis, is rare among the natives. However, in spite of conflicting data, they apparently believe that excess copper may be harmful; although copper may have its use, its too general may be harmful.

In connection with an investigation in which he determined that copper is apparently a constant constituent of urine of normal individuals, Rabinowitch (105) stated that, although copper may be an essential element of biological material, there is reason to suspect that exposure of the human body to excess quantities may do harm. He also called to attention that, in the interpretation of urinary data, consideration must be given to the fact that the differences between health and disease are quantitative and not qualitative.

Beijers (132) describes cases of copper poisoning in sheep grazing in orchards where a CuSO_4 spray had been used. The liver and kidneys showed degenerative changes, and large amounts of copper were found in the liver and bile.

Luchsinger (112), in 1951, reported two cases of laboratory workers affected by the caustic action of finely ground copper on the mucous membrane of the nose and throat. He stated that these cases deserved the attention of the accident physician as well as of the general practicing physician because they indicate that copper in metallic form and as copper salts can cause important local caustic effects.

Also, owing to their solubility, they diffuse in the body, are eliminated through the kidneys, and can be detected in the urine.

Elkins (133) stated that:

Although copper salts are toxic, especially to lower forms of life, and copper is widely used in industry, industrial copper poisoning is virtually unknown. This fact is undoubtedly due in part to its relatively low toxicity to man, and in part to the low volatility of copper and its compounds.

There have been a few reports of chronic copper poisoning among metal workers, but these have not been substantiated. In its ordinary applications in industry, copper may be considered virtually harmless.

In a paper on Copper Metabolism in the Invertebrates, presented by Dethier (134) in 1950 at a Symposium on Copper Metabolism, death is said to ensue when the copper concentrations to which an organism is exposed are increased beyond the capacity of the excretory organs to eliminate the material. Although the physiology of copper metabolism and its mode of action as a poison are even now not completely understood, it has been realized for a long time that copper compounds are effective killing agents (134).

TREATMENT OF COPPER POISONING

Little consideration is given in the literature to treatment of copper poisoning. Except in attempted suicide, acute copper poisoning is rare, as toxic doses are so large that they are immediately rejected by the stomach (66), and involuntary injection of copper compounds is unlikely due to the peculiar metallic taste, which serves as a warning of danger (22); chronic poisoning probably never occurs, because when the minimal dose is exceeded acute symptoms occur (15).

According to Preble (26), in doses of 10 or 15 grains copper salts act as an emetic, a property that serves a conservative purpose through rejection of the poison when larger doses are taken. The treatment recommended by Preble is to wash out the stomach; if medical aid is not available white of egg and milk may be used as first-aid antidote. Ferrocyanide of potassium is the chemical antidote and may be added to the lavage water. Collapse should be treated in the usual manner by warmth, heart stimulants, and similar measures, and rectal feeding may be required. In the table of cases of poisoning prepared by Lehmann (58) from the literature, methods of treatment mentioned are venesection, use of stomach pump, and administration of aperients; in most instances, however, vomiting followed immediately after ingestion of the copper. Sternberg (120) recommended lavage and white of egg for treatment of acute poisoning by copper salts and finely pulverized iron or iron compounds for the chronic form, as the iron, according to him, attracts the copper out of its combinations. Eisner (29) healed dermatitis caused by copper dust by desensitization with copper sulfate salts.

Filehne (31) believed that breathing fine copper powder presents a hazard, the limiting of which for the workers is well worthwhile. He recommended as a preventive measure that the workers gargle and thoroughly clean their hands before eating.

Investigations of the biochemistry of BAL, cited by Lusky, Braun, and Lang (135), revealed that copper greatly accelerated the oxidation of BAL when used as a catalyst in vitro. A high level of copper excretion could be maintained over long periods in normal animals by administering BAL-Intravenous at 2-hour intervals. An

investigation was made to determine the therapeutic efficacy of BAL in experimental copper poisoning by a 1 percent aqueous solution of cupric sulfate.

The average survival time of 15 rabbits after the intravenous administration of 20 mg. per kilogram of cupric sulfate was 15 minutes. The effect of BAL treatment was spectacular. The treated animals received a single intravenous injection of BAL, 30 mg. per kilogram, within 3 minutes after the administration of the cupric sulfate. Only 1 of the treated rabbits died, and the life of that animal was prolonged 32 hours. Three rabbits that received 20 mg. of cupric sulfate per kilogram intravenously were saved by single intramuscular injections of BAL in oil, 30 mg. per kilogram.

In several experiments the right carotid artery and the trachea were cannulated and the blood pressure and the respiration of the rabbit recorded on a kymograph. After cupric sulfate was administered intravenously, 20 mg. per kilogram, the blood pressure fell steadily, the pulse became sluggish, and there was a corresponding decrease in rate and amplitude of respiration. In approximately 15 minutes, as the blood pressure approached 0, no pulse was recorded, and respiration ceased. BAL administered intravenously, 30 mg. per kilogram, caused an immediate and sharp rise in blood pressure, the pulse became rapid and strong, and, as the blood pressure approached normal, the animal began breathing again.

COPPER DEFICIENCY

Effects on Plants

According to Arnon (136), a clear demonstration of copper as an essential micronutrient for the growth of higher plants was presented by Sommer in 1931. However, the early evidence for the need of copper by higher plants did not gain prompt acceptance as a new essential micronutrient. Considering the desirability of confirming its specificity and of excluding the possibility that some other element capable of existing in several valence states could replace copper, Arnon and Stout (137) reexamined the status of copper by testing its essential nature. An element was not considered essential unless:

- (a) A deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stages of its life cycle;
- (b) such deficiency is specific to the element in question, and can be prevented and corrected only by supplying this element;
- and (c) the element is directly involved in the nutrition of the plant, quite apart from its possible effects in correcting some unfavorable microbiological or chemical condition of the nutrient medium.

Using the tomato as a test plant, it was found that copper satisfied these criteria of essentiality. The findings showed the direct effect of copper on the plant as distinguished from its possible effects on the root environment and were interpreted as providing the final link in the chain of evidence of the essentiality of copper for plant growth (136).

The symptoms of copper deficiency in plants do not constitute a uniform pattern in diverse species but show a distinct pattern for a given species. In the test, under controlled conditions, on tomatoes (136) the symptoms were: Very much stunted growth of shoots and roots, curling of leaves, failure to produce flowers, and a bluish-green color of the foliage, with a sheen very distinct from any other deficiency symptoms observed in that plant.

Although no plant growth is possible without a supply of copper, due caution must be exercised in providing a concentration in the nutrient medium compatible with good growth yet avoiding a toxic level. For the tomato 0.02 p.p.m. in the nutrient solution was adequate, but a concentration of 2.0 p.p.m. was distinctly toxic (137).

Arnon (136) quotes numerous investigators (52 references are cited) in his discussion of the various functions performed by copper in the metabolism of plants.

Steinberg (138) described various methods for effective removal of trace-element impurities in plants, quoting from the work of numerous investigators on this subject and on the influence of minute quantities of copper on the nutrition of an organism (85 references are cited).

Thompson (139) has described reactions that indicate that trace amounts of copper in foods are bound in proteins and can result in a browning reaction, the mechanism of which differs from known types of enzymatic browning. Certain findings indicate unusual properties of complexes formed during the copper-protein browning. Copper protein is defined as protein that has reacted with copper and has been washed under normal conditions to be free of residual copper. Methods used to distinguish between bound and free copper were discussed at the Symposium on Copper Metabolism when Thompson (139) presented a paper on A Browning Reaction Involving Copper-Proteins. The catalytic effect of copper also was considered by Thompson (139).

Effects on Animals

Dethier (134) called attention to the number of species of invertebrates that have been analyzed for the presence of copper since that element was demonstrated more than 100 years ago to occur in animals. The number is so large that a "tabulation of data from all sources would be prodigal in the extreme" (78 references are cited).

In certain areas, where phosphorus is apparently present at normal levels in the feed, cattle are unable to form bone properly without copper supplements. Without the copper a condition similar to rickets occurs in young calves, while in older animals osteomalacia (softening of the bones) develops. The abnormality in bone formation is evidenced by broken bones and enlarged joints, but other evidences of malnutrition are apparent. Davis (140) cites as indications of malnutrition low hemoglobin values, emaciation, achromotrichia (graying of hair), stiffening in the joints, erosion of the joints, and occasionally abnormal gait sometimes called "paces" from resemblance to the pacing gait of a horse. The texture of the haircoat may become rough, sores that develop in the skin fail to heal normally, and the animals may die unless moved to a different, unaffected pasture or given some supplemental feed, such as cottonseed meal, from a different area. Copper sulfate administrations in large measure corrected the difficulties and served as a base for experimental work on the influence of copper on the metabolism of cattle.

Calves showed the changes described (140) more rapidly than older animals. Within 60 days the ends of their long bones showed swelling, there was beading of the ribs, and a typical rachitic picture appeared to be developing. In older animals the copper deficiency was somewhat slower in appearing; the usual first indication was loss of weight, beginning achromotrichia changing the dark red Devons to light tan, and lowered hemoglobin values.

In discussing the effect of copper-deficient fodder on sheep, Marston (141) stated that, in the course of the relatively slow, progressive depletion of the copper reserves that occurs in mature animals when they are confined to copper-deficient rations, an ordered sequence of distinct symptoms appears, as if, 1 by 1, different metabolic processes become adversely affected, each in its turn, through partial impairment of the particular enzyme system that governs it.

Marston (141) mentions more particularly the process of keratinization (becoming horny), a lesion in the wool being the first sign of impaired function in copper-deficient sheep. The changes in structure of the fiber probably results from a breakdown of catalytic processes in which copper is primarily involved.

Cunningham (142) describes "peat scours" affecting cattle and sheep in New Zealand. At first it was believed that the scouring was caused by internal parasites. The first hint that a copper deficiency might be involved came from observation that bluestone and nicotine sulfate drenches afforded temporary relief to scouring cattle. The hypothesis was formed that the beneficial factor was copper. Diseases in cattle and sheep in some regions of New Zealand result from a quantitative deficiency of copper and in others from a copper deficiency, which is complicated and in part induced by an excess of molybdenum in the food. Simple deficiency in cattle is characterized by lack of thrift, anemia, and brittleness of bones of young animals; complicated deficiency is indicated by these symptoms together with a severe seasonal diarrhea. Deficiencies of both kinds in sheep produce symptoms only in lambs, which develop osteoporosis (porousness of bone) or atoxia.

According to Cartwright (143) no uncomplicated or clearly defined copper deficiency has ever been reported in human subjects. A combined deficiency of copper and iron has been demonstrated in an occasional child with nutritional anemia. Cartwright (143) summarizes the literature on copper therapy by saying that most investigators agree that in infants and young children hemoglobin regeneration is accelerated when copper is given in addition to iron. In adults supplemental copper therapy is unnecessary and of no demonstrable value in most patients. It is not surprising that a deficiency of copper has not been demonstrated in adults, as the daily requirement of 2 mg. is easily supplied by even a mediocre diet and as there is storage of copper in the tissues. (72 references are cited.)

COPPER IN TREATMENT OF DISEASE

Diseases in General

As mentioned previously, Pliny (13) gave a rather detailed description of the use of copper in medicine, which included a number of prescriptions and instructions for their preparation. No doubt much of his material on this subject, as on other subjects, was collected from earlier writings, indicating the antiquity of the use of copper as a medicament. The following statement is taken from Bailey's translation of Pliny's Natural History (13):

The healing art profits much from copper mines, for nowhere do ulcers heal more quickly than in their neighborhood. Cadmea is the most effective agent. The latter unquestionably occurs also in furnaces used for silver smelting, but it is a paler and lighter variety, far inferior to that obtained from copper ores. The name cadmea is used to indicate more than one substance - firstly, the actual mineral from which the metal is prepared, indispensable to the copper-smelter and not without its use for the physician;

secondly, a different substance, formed in the smelting-furnace and taking its name from its method of production.

All cadmea, the best of which comes from the furnaces of Cyprus, is reheated by the physicians with clean charcoal and, when it has been reduced to ashes, is quenched with Aminean wine or vinegar, according to whether it is destined for the preparation of plasters or for the treatment of itch. Some authorities heat the pounded material in earthen vessels, wash it in a mortar, and dry it. Nymphodorus recommends choosing as heavy and compact a mineral as possible and heating it with live coals. Then he quenches the ignited material with Chian wine, pounds it, sifts it through a linen cloth, pulverizes it in a mortar, macerates it in rain water, and subjects the part which settles quickly to a further pulverization until the consistency of the material resembles that of white lead and no grittiness can be detected by the teeth. Cadmea acts as a drying and powerful healing agent. It checks discharges, clears up films and incrustations of the eyes, removes roughnesses, and has the general effects related in the case of lead.

Copper, too, is roasted for all the same purposes, as well as for scars and white spots on the eyes and for healing ophthalmic ulcers, when applied in milk. It is ground, after the manner of Egyptian salve, in a small mortar. Taken in honey, it promotes vomiting. "Flower" of copper also has medical uses. It is prepared by fusing copper and transferring the molten metal into a second furnace. There, by increasing the blast of air, "flower" of copper is driven out on to the surface like so many husks of millet. Scale and "flower" of copper are alike roasted in flat vessels of earthenware or copper, then washed as above for the same purposes. Furthermore, they remove fleshy growths of the nostrils and the anus, and cure deafness if blown into the ears through a reed. They reduce swellings of the uvula and tonsils, when applied in powder form and with honey, respectively. From "white copper" is made a scale far less effective than that from Cyprian copper. Some macerate the nails and cakes of copper in the urine of a boy before the scale is hammered off, after which the latter is powdered and washed with rain water. Dropsical patients are treated with two drachms in a half-pint of mead, and it is also used with fine flour as a liniment.

Another widely-used substance is verdigris, which is obtained in several ways. Verdigris is washed like cadmea. Its special utility is as an eye salve, and for inducing tears by its pungency, but it must subsequently be washed off with a sponge dipped in warm water until the corrosive action ceases. The name hieracium is given to a salve which is generally as follows: Four ounces of gum of Hammon, two of Cyprian verdigris, two of shoemakers' black (also called chalcantion), one of misy (copper pyrites, oxidized pyrites, containing CuSO_4 and FeSO_4), and six of saffron. This mixture is ground with Thasian vinegar and made into pills, used with good effect in the initial stages of glaucoma and cataract, against films or scabs of the eyes, leucoma, and diseases of the eyelids. Crude verdigris is used as a constituent of wound plasters. Ulcerations of the mouth, gums, and lips show wonderful improvement when it is applied in oil,

but if it is applied as an ointment in wax it cleanses the ulcer and induces the formation of a scar. It removes the callous skin from fistulae and abscesses about the anus, if rubbed in by itself or with gum of Hammon, or pressed into the fistula like a suppository. Made up with a third part of turpentine resin, it removes leprous scales.

The name chalcitis is given to another mineral from which copper is smelted. It differs from cadmea in that the latter is hewn from exposed rocks on the surface of the ground, while chalcitis is mined from deeply buried deposits. Again, chalcitis is soft by nature, like condensed wool, and crumbles quickly. Another point of distinction is that chalcitis contains three constituents, copper, misy, and sori, to be described each in its proper place. It is best if it is honey-colored with a delicate network of veins, friable, and not hard. It is supposed to be better when freshly mined, for as it ages it turns into sori. It is used for ulcerous excrescences and arresting hemorrhage, as a powder to act astringently on the gums, uvula, and tonsils, and as a pessary in wool for diseases of the uterus. It is made up with leek juice into pessaries for diseases of the genitals. The sori of the best repute comes from Egypt, and is far superior to Cyprian, Spanish, or African, although some prefer Cyprian for the treatment of diseases of the eyes. Whatever the source, however, the best sori is that which has the strongest smell and which, when ground up, has an oily black color and a spongy consistency. It is so offensive to the stomach that, with some people, the mere smell of it causes vomiting. Such is the Egyptian variety. Sori from any locality sparkles like misy when broken up and is of a harder consistency. It cures toothache if it is put into the cavities and brought into thorough contact with the teeth, and it heals noxious and serpiginous ulcers of the mouth. Like chalcitis, it is ignited on a fire of coals.

Misy is prepared by boiling 16 drachmae with a hemina of vinegar and some honey until the mixture thickens. Whenever it is thought desirable to moderate its strength, a little honey is added. Used as a fomentation with vinegar, it removes the callosities of fistulous ulcers, and it is employed as a constituent of unguents. It stops bleeding, arrests the development of serpiginous and putrefying ulcers, removes fleshy excrescences, is specific for diseases of the male genital organs, and checks menstruation.

The atramentum (copper sulfate?) from Cyprus is the kind most highly prized for medicinal purposes. A dose of one drachma is taken in honey to expel intestinal parasites. If dissolved and used as a nasal douche, it clears the head, and it acts as a purgative if taken with honey or honey water. It is a remedy for granulations of the eyelids, pains in the eyes, dimness of vision, and ulcerations of the mouth, and it checks bleeding from the nose and from hemorrhoids. Used in combination with henbane seeds, it helps splinters of broken bones to work out, and it arrests watery discharges of the eyes if applied to the brow with a sponge. It is an active ingredient of plasters used for cleansing wounds and ulcerous excrescences.

According to Lehmann (58), Hoenerkopf in 1855 supplemented Paasche's critical observations and Rademacher's investigations on the harmlessness of taking copper oxide for long periods by therapeutic experiences with large doses of copper administered to children suffering with the croup. Ninety-one children with croup received copper sulfate in single doses of 0.06 to 0.3 gm., at first every 10 minutes, then every 15, 30, and 60 minutes, so that sometimes in severe cases 1.08 to 1.44 gm. copper sulfate was used during the course of an hour. The aim was to cause vomiting with each dose, and this result was attained with few exceptions. The largest amount a child received in succession was 12.86 gm. in 8 days, that is, an average of 1.62 gm. per day. The child died with the croup.

The therapeutic value of copper in the treatment of croup is not discussed in the summary of Hoenerkopf's investigations as given by Lehmann with the exception of a statement that the administration of copper ceased only with the cure of the croup.

Copper compounds were recommended for their antiseptic properties. Miquel (144) in 1883 called attention to the very high rank of copper sulfate among compounds having the power of preventing or delaying the putrefaction of animal substances, being 2 or 3 times more antiseptic than the salts of lead, uranium, thallium, nickel, zinc, aluminum, cobalt, and manganese. However, he said it had to give precedence to the soluble compounds of platinum, gold, silver, and mercury but had an antiseptic action a little superior to that of salicylic and benzoic acids, with twice the disinfectant power of thymic acid, 3 times that of phenic acid, 5 times that of alum, tannin, arsenious acid, and 10 times that of chloral hydrate and the salts of protoxide of iron, and the soluble haloids, as cupric chloride, had still greater disinfectant properties. He concluded that repudiation by surgery and hygiene of cupric compounds deprived them voluntarily of powerful auxiliaries having the advantage over many other compounds of being very low priced and easily handled.

Marry (145) had the following to say regarding the use of copper sulfate:

When any reason whatever militates against the use of sublimate, copper sulfate can be considered as the strongest antiseptic that can be used with absolute safety. A hundredth part solution of this salt acts with sufficient energy.

In addition to its greater antiseptic power it should be preferred to phenic acid solution because it does not cause irritation of the wound and the burning sensation produced by the latter when used with the high titration that only an absolute antiseptic reaches. It should also be preferred because it has no odor itself and takes away all fetidity of the lochia and, consequently, the bad odor from the room. Intrauterine injection is absolutely harmless and its coagulative action could be utilized in case of uterine hemorrhage. The use of copper sulfate externally causes no poisonous symptoms. Internal use should be delayed for the present. The facts authorize the statement that copper sulfate constitutes a medicine for local application of the first quality with which surgery can obtain the best results in the care of wounds.

Charpentier (146) drew the following conclusions from the use of copper sulfate in obstetrics:

1. Copper sulfate used in a hundredth part solution is an antiseptic of the first order and can render signal service in obstetrics;

2. Absolutely inoffensive to the patient, moderate in price, easy to handle, it has the added advantage of being a very powerful antiseptic, that is to say an instantaneous disinfectant;

3. Whether used in the form of intravaginal or intrauterine injection it is absolutely harmless;

4. Copper sulfate enjoys astringent and coagulating properties such that it could be substituted as a hemostatic for perchloride of iron, over which it has the superiority of not staining the wound;

5. The solution should be one hundredth part heated to a temperature of 36° to 38° ;

6. The solution can be used during the first 8 or 10 days with several repetitions during the 24 hours without other effect on the patient than lowering of the temperature and diminution of the pulse frequency, that is, a rapid and incontestable improvement;

7. Surgeons have every interest in trying this antiseptic which, in certain instances, in particular in thrombus of the vulva, healed without a drop of pus. In a case of fetid abscess it stopped immediately the fetidity and the general symptoms resulting from the infection when phenic solutions had failed.

In experiments carried out on dogs and rabbits at the University of Gand, Du Moulin (147) administered without harm at one time 4, 5, and 6 gm. of copper sulfate to dogs; these animals vomited, but some hours afterward all were well. Subacetate of copper or verdigris was administered every day for 6 weeks in doses of 1 to 2 gm. without producing any toxic symptom. At first the animals vomited, but recurrence of this symptom ceased at the end of 4 or 5 days. The oxide and the carbonate of copper were also administered in progressive doses for a year to rabbits, which never failed to grow large and strong.

Du Moulin (147) did not stop with experiments on animals; encouraged by those made without any inconvenience by some practitioners on man (advice of Dr. Galippe), he prescribed for infants affected with impetiginous eczema 12 to 15 cg. per day of copper sulfate, a medicine that had cured a young dog of a similar affection. All the infants recovered without manifesting any sign of poisoning. He treated in the same manner patients with ophthalmia, ganglionic engorgement, and scrofulous osteomyelitis without noting any irritation. Finally, he prescribed for infants suffering with the croup potions containing 40 to 50 cg. of copper to be taken by the spoonful every half hour, then at intervals of 1 to 2 hours for 4 or 5 days; the infants recovered from their dangerous affection without the least sign of poisoning. From these experiments Du Moulin concluded that copper salts administered by mouth in proper doses are not toxic.

In 1925 Zavalia (148) reported results of the treatment of 50 cases of trachoma with 2 solutions: (1) Copper sulfate 0.10 gr., novocaine 0.40 gm., and distilled water, 10 gm.; and (2) novocaine 0.40 gm., adrenalin xx drops (1-thousandth part), and distilled water 10 gm. Of the 50 cases treated with these solutions, 28 (58.3 percent) were cured; 12 (25 percent) were notably improved; 3 (6.25 percent) were slightly improved; and 5 (10.4 percent) showed no effect. Painful reaction edema resulting from the injection reached exaggerated proportions, and the treatment was abandoned in only one case. The author concluded that conjunctival injections of copper sulfate constituted an efficacious enough method for the treatment of conjunctivitis granulosa if used according to correct technic, were harmless, and

were very easy to apply. Incipient trachomas were cured completely and promptly. More severe cases were favorably affected but more resistant. The effect of the treatment on corneal ulcers was rapid and satisfactory.

Curtis and Scott (149) had the following to say in regard to the general medicinal properties of compounds of copper:

As is usual in the case of compounds of the heavy metals, impregnation of the blood with a copper compound tends to affect nutrition. In medicinal doses too small to produce any obvious derangement, the influence tends to the abatement of spasmodic nervous disorders, but not to a sufficient degree to enable copper to compete with zinc and other remedies as a practical antispasmodic. In excess of medicinal dosage copper salts are readily poisonous.

Locally, soluble copper salts are powerful irritant astringents. Applied in concentrated form to a moist surface they combine with the albuminous elements of the part, forming a thin, practically invisible slough, and at the same time constrict and irritate. As far as the caustic effect is concerned, it is so superficial as to be of little moment, so that practically the action is simply conjoint astringency and irritation. Such action, however, may determine absorption of easily absorbable tissue, such as granulations, or the healing of indolent ulcers, or the abatement of catarrhal process. Taken internally, in any beyond quite small dosage, the irritant action of soluble copper salts declares itself by producing at once full and free vomiting. Such vomiting is attended with little nausea or depression, and the emetic dose is itself discharged with the ejecta. Copper salts thus become available medicinally as emetics, and because of their power and promptness of action are peculiarly appropriate for the removal of poisons by vomiting. In considerable single overdose copper salts are irritant poisons.

A single compound, only, of copper is official in the United States Pharmacopoeia, namely, the sulfate (cupric sulfate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$). The salt is entitled Cupri Sulphas, Copper Sulfate, and is the one so well known by the common name of blue vitriol or bluestone. It occurs in large, transparent, deep blue, triclinic crystals; odorless; of a nauseous, metallic taste; slowly efflorescent in dry air. Soluble in about 2.2 parts of water, 400 parts of alcohol, and 3.5 parts of glycerin at 25°C (77°F .), and in 0.5 parts of boiling water (U.S.P.). The salt effloresces slowly on exposure, or on moderate heating, to an opaque, pale blue powder. Cupric sulfate may be used locally in substance, a smooth crystal, with its edges rounded by a file, being selected, or cylindrical or conical pencils may be used by fusing one part of alum with two of the copper salt and shaping in suitable moulds. Lotions are made by aqueous solution, the strength ranging from one half to 2 percent. As an internal astringent the dose is about one fifth grain (0.01); as an emetic gr. iv. (0.25) or more if the stomach is insensitive from narcotic poisoning. But if a full dose is given, and fails to produce emesis, it must be removed by

stomach tube lest undue gastric irritation result. The salt may be given in powder, mixed with powdered sugar, or in a 1 percent solution. It is particularly valuable in phosphorus poisoning for in addition to its emetic properties, it forms an insoluble phosphide of copper, and so prevents further absorption.

Treatment of Cholera

In 1867 Burq (150) published a book on the results of his investigations in regard to the immunity to cholera of copper workers. This was the beginning of a controversy on this subject, which raged especially during 1883 and 1884. In this book he called that Paracelsus used copper, among other metals, in treating disease. Soon after the death of Paracelsus, Pomme became celebrated in the treatment of nervous diseases by long-continued baths. An abbé, in a note lost at the bottom of one of his pages, advised that prolonged baths were more effective when taken in copper vessels. Neither Pomme nor his imitators paid any attention to this and many doctors were astonished not to have the same success as Pomme, without knowing why they did not.

Burq also mentioned that a sort of diadem of yellow copper was worn by the women of Arctense, who considered it efficacious in preventing headaches, which were very frequent in that cold and humid country. He also quoted Hannemann as saying that in Hungary it was thought that wearing a thin piece of copper against the skin protected against cholera.

Burq (150) describes an intensive investigation carried out by him in 1853 in Paris and other French cities and in foreign countries to determine the immunity to cholera acquired by workers in copper. In a copper smelter in Paris he found that about 200 workers had been "respected" by the cholera in 1832, 1849, and 1852. The same was true of between 400 and 500 workers in 3 other smelters on the same street. He visited about 400 establishments in Paris, all of industries in which copper was used, from the most modest shop employing only 4, 5, or 10 workers up to the great establishments employing hundreds. He received reports from England, Sweden, and Russia on a population embracing over 200,000 individuals, in accordance with which he presented the following conclusions to the Academies of Science and Medicine:

In the cholera epidemic, constant preservation of an immense majority of workers whose occupations required habitual exposure to copper was observed.

Copper or its alloys, brass and bronze, applied liberally and permanently to the skin in the cholera epidemic are effective means of prevention which should not be neglected, especially since there can be no inconvenience in their use and, if the relative prophylaxis which apparently should result from them should happen to leave something to be desired, perhaps it would be useful to assist by purchases of very fine powder of this material and as a last precaution large sheets of brass might be concealed in the homes of well-to-do people.

In the treatment of cholera, copper administered at the proper time, either alone or associated with agents such as opium which have the sanction of experience, or in the form of filings or some other form in which the actual dose and best method of administration can be determined without delay, has the greatest chance of becoming in the hands of physicians a powerful means of healing.

Burq said the indication was to put everyone as completely as possible in the same condition of copper absorption as the workers who had been completely preserved from the cholera. He was struck with the fact that persons in certain occupations who worked alone or in a room or small shop had only a relative protection against the cholera on account of the small concentration of the metal in the air, whereas those who worked in the vicinity of other workers who threw finer and larger quantities of copper dust about them enjoyed a common immunity. At one large electrometallurgical plant he found there had not been a single case of illness from cholera, whereas two men in the personal service of the owner-director died of the disease. The latter, struck by this fact and by others, wrote to Burq that more than ever he believed in the efficacy of copper and its compounds as certain prophylaxis against cholera.

Burq quoted reports from persons, such as physicians and mayors, of several cities in Italy and Spain to the effect that no cases of cholera appeared among the copper workers there. He emphasized that workers in copper who are immune to cholera are those who are permanently exposed to absorption of the metal. In the 1865 epidemic in Paris, of the 1,667,000 persons composing the population within the walls of the city 6,176 were victims of the cholera, an average of 3.7 per 1,000. About 30,000 were engaged in the various copper industries, and of this number 45 died, an average of only 1-1/2 per 1,000. But, according to Burq, if the figures were deducted for those who were not exposed to much if any copper in their work, the number could be reduced from 45 to 10 or 12, an average of 1 in 3,500.

Burq cited a number of cases of cure of cholera when the patients were at the point of death and described how he protected himself from the cholera by wearing pieces of copper touching the skin and by lavage with a solution of copper sulfate. However, he claimed to have had a slight attack while investigating the disease in the epidemic at Toulon. For treatment he recommended to take, according to age, once or even twice a day in a little sweetened water 2, 3, 5, or 6 drops of a solution of 5 gm. of acetate or sulfate of crystallized copper, 1 gm. laudanum, and 20 gm. of ordinary water. Each drop he stated represented 1 cg. of salt. The chief medical officer of the insane asylum at Marseille, in a statement quoted by Burq, cured cholera in several persons by using 5 gm. of copper sulfate and 100 gm. of distilled water in a potion composed of 1 gm. 50 cg. of the copper sulfate solution, 10 drops of laudanum, and 120 gm. of sweetened water. He thought Burq's doses were too strong.

As Burq claimed that in cases in which the copper was ineffective it had not been absorbed, Guichard and Duriez (151) under his direction examined the livers of 6 persons who had died of the cholera after receiving 2 and sometimes 3 doses, each containing 40 cg. of sulfate of copper and 1 or 2 lavages with 30 cg. of the same salt. Each liver was boiled in pure nitric acid and then treated with sulfuric acid. From the result of the experiments, in which they found copper, they concluded that in the treatment of cholera with this metal lack of success was not due to the nonabsorption of the copper, since they had demonstrated chemically that it had been absorbed. They considered the immunity from cholera of workers in copper smelters or workshops to be very doubtful.

In 1883 Davin (152) called the attention of the Academy of Sciences to a memorandum that he had addressed to the Academy in July 1873 on the efficacy against cholera of the bronze powder used by gilders. He had recommended at that time that this powder be scattered in workrooms with a bellows and cited various examples in which this precaution had protected those who had had recourse to it from the cholera.

In 1883, it appeared to Burq (153) that the time had come to present a summary of the results of this long study, which he had been carrying out for more than 30 years

and the deductions to be drawn therefrom on the action of copper against the cholera. He stated that:

Observations and facts without number, verified and often openly attested by all those who in France, Switzerland, Russia, Germany Spain, Italy, and even in the very cradle of the disease, Bagdad, have taken the trouble to consider carefully, which have never been denied by any ocular witness; statistics of death from cholera prepared by Trebuchet and by Blondel; an inquest by the prefecture of police after the epidemic of cholera in 1865-66; a report by Michel Levy in 1861, by Vernois in 1869, and by Devergie in 1876 to the Council of Hygiene and Health; a fourth report by Dr. Pauchon to the Society of Medicine of Marseille in 1873, have demonstrated that all the individuals who, through their occupation, cohabitation, or even simply through proximity to the copper industries, suffer daily impregnation by copper generally enjoy in respect to the cholera a proportionate immunity, and the exceptions - and they exist - are at least as rare as smallpox is among persons well vaccinated. At the head of the immune are all the workers in whom the coloration indicates a sort of copper saturation, such as the makers of copper musical or optical instruments, dry polishers, lath workers, the real coppersmiths and not the tinsmiths, the repair men, the founders, the bronze workers, etc.

Burq said this preservation was not surprising in the light of recent experiments that had placed copper high in the ranks of antiseptic materials that could be used on man without danger and the fact that the Society of Medicine of Paris and the Council of Hygiene had made copper salts one of the principal bases of hygienic prescriptions. From experiments he had made in 1869 and later confirmed by Galippe, Burq said it was known that copper could be ingested with impunity in the quantity necessary to attain the same condition of impregnation as that of the most storing workman. He called attention to the use by Maillet in Japan of waistbands of copper for large-scale prophylaxis and said that even at the moment, according to Vulpian, at Mekong in the delta of the Ganges and in Egypt the French and English officers were protecting themselves with copper. Burq summarized his recommendations as follows:

The external application of copper, either in the metallic form - armatures, plates, or even ordinary sous after having been well scraped and sewed on a supple leather thong - or by tincture (dye) in a vest, chemise, or flannel waistband;

The burning at home of bichloride of copper in the alcohol lamps;

Daily use of preparation of copper in protective doses, for example bioxide, which has no taste, beginning with 0.01 gm. up to 0.06 gm. according to age twice a day, or wash with warm water containing 0.10 to 0.20 gr. of copper sulfate;

Add natural mineral water of Saint-Christan to the wine at meal time, make use of vegetables made green with copper sulfate;

Conform especially to the sensible hygienic precautions published by the authorities.

These are, according to Burq's experience, the efficacious methods of preservation against cholera.

Later, a letter from Vulpian (154) denied responsibility for the statement accredited to him by Burq. Vulpian said that Burq without doubt had taken this information from a newspaper that had attributed to him (Vulpian) by error part of an article devoted by one of its editors to the study of methods of protection against cholera. It was this editor who had made Vulpian acquainted with what someone had told him of certain preventive practices put into effect in Cochin China, India, and Egypt, but Vulpian himself had never heard of it before and did not wish to take any responsibility regarding the use of copper in the prophylactic treatment of cholera. The value of the treatment appeared to him to be very doubtful: If copper had been really efficacious probably it would have been used long ago in all countries where cholera occurred, especially in parts of India where this disease is endemic. He did not, however, know of any inconvenience in the attempt, with moderation, to protect against cholera with the aid of copper, on the condition that no one was caused by too great confidence in a probably chimeric method to put aside the hygienic prescriptions that should hold the first place for the moment in the prophylaxis of cholera.

Brame (155) sent a note from Westminster relative to an example of immunity from the cholera that he considered as attributable to the action of copper. The town of Fahlun in Sweden, in the vicinity of copper mines, had always been free from cholera. He thought that the vapors released into the air by the metallurgical operations, which rendered impossible the growth of all vegetation of the surrounding hills, should also have the effect of destroying the germs that served to transmit the cholera.

Later, Vulpian presented the following note by Axel Lamm, Stockholm (156):

In the spring of 1853, the cholera being present in Europe, the Swedish public under the impression of the prophylactic decrees of 1830 and the painful recollections of the first epidemic of cholera in Sweden (1834) desired to have something tangible to use against the infection. Magnus Huss, then head of the medical clinic at the hospital of the Seraphins, in Stockholm, advised to carry on the pit of the stomach small plates of metallic copper. This was suggested to him by the fact that the cholera had not appeared at Falun, principal city of the Delarne Province (Dalecarlie), in proximity to the considerable copper mines which have been worked since the most remote antiquity and in part in the open air. The use of the plates (which were thin and round, about 0.10 m. in diameter) gave no results from the point of view of prophylaxis against the cholera.

It happened, on the other hand, that in certain cases, due to the effect of transpiration and because care had not been taken in drying the plates, there formed on their surface verdigris which acted as a caustic on the skin when in direct contact with it, causing ulcerations.

Lamm stated that it was true that the cholera, which had appeared 5 or 6 times in Stockholm, had never penetrated into the north as far as Falun, but said it was well to observe that at Falun and around Falun the oxidation of the minerals emitted into the atmosphere sulfuric acid gas in quantities at times intolerable. He asked the question as to whether this circumstance exerted an influence on the miasm of the cholera epidemic but said

he was going to content himself with asking but was not going to consider it here, not having the necessary facts to arrive at a solution.

In discussing the documents collected by Ygouf and transmitted by Bochefontaine to the Academy of Sciences (19), the latter stated that if copper conferred immunity from the contagion of microbic or zymotic diseases, then the workers at Villedieu, who were so saturated with copper that it was the normal condition, would have been protected from these diseases. However, there were 9 deaths from the cholera in Villedieu in 1849, some of them among copper workers or their families; if the population of Villedieu had been as large as that of Paris, everything else being equal, the mortality from the cholera would have been 5,700. Bochefontaine quoted from a letter received from the Mayor of Villedieu to the effect that there had been no case of cholera among the inhabitants of Villedieu since 1852 although in 1848-49 several workmen continually in contact with copper died of cholera, but this was cited as entirely exceptional. Apparently the incidence of smallpox, typhoid fever, and anthrax was not affected by the copper.

Bailly (157), an industrial doctor who had practiced medicine for 16 years in the village of Bornel, where more than 500 workmen were employed in making tableware and other objects of the goldsmith's art from alfenide metal composed of copper, tin, and zinc, in which the proportion of copper was 70, 80, and even 90 percent, found that copper compounds did not protect the workmen against typhoid fever, measles, mumps, whooping cough or anthrax, and did not exempt from the choleriform diarrhea. His statements were referred to the commission appointed to investigate the work of Burq.

Burq (158) replied to the above and other criticisms in several succeeding issues of the same magazine, in which he repeated more or less what he had published previously and quoted physicians and others whose statements contradicted Bailly's claims and proved his own contention regarding the efficacy of copper as a prophylactic agent against other infectious diseases besides cholera.

Bordier (159) entered the controversy with the following statement regarding Burq's contention:

Burq has recently presented some new facts to show the immunity that copper confers for certain diseases. For a long time he has put all his perseverance at the service of this idea which has only met with incredulity; and still today many doctors refuse to see anything but a coincidence in the remarkable figures by which this conscientious observer has shown that in the last typhoid fever epidemic in Paris the workers who handled copper had presented, all things being equal, an incontestible immunity.

Moreover, what was difficult to comprehend some years ago today seems more natural; Burq probably did not suspect, when 20 years ago he undertook his campaign to demonstrate the pathological immunity conferred by copper, that Pasteur would some day be his most useful ally. The researches of Pasteur have led to a comprehension of a multitude of phenomena that otherwise would have remained inexplicable. Returning to the statements of Burq, the works of Pasteur make comprehensible that a certain quantity of copper in the humors make them a poor culture medium for the microbe of typhoid fever.

In still another article Burq (160) returned again to the subject of copper in infectious diseases. He addressed himself in Paris and elsewhere to all the

great industrialists who worked copper and bronze and confirmed everywhere an equal immunity for typhoid fever as for cholera and even smallpox. According to him the continual absorption of copper, when nothing intervenes to annul the effects of this impregnation, ends by modifying the economy in a manner to render it unsatisfactory for the culture of certain morbid germs. He concluded by saying that proof was ready for cholera and typhoid fever, was being investigated in connection with smallpox and diphtheria, and would be proved for other diseases, such as yellow fever.

Vallin (161) quoted a remark by Bouley that Burq's hypothesis regarding the prophylactic value of copper in cholera could find support in certain new facts with reference to the efficacy of salts of certain metals in diseases, as sublimate in syphilis, and arsenic and quinine in malaria. He stated that these hypotheses were seductive but that new investigations made by various authors in different countries had not confirmed the immunity from cholera of workers in copper that Burq believed he had demonstrated by his personal investigation. Vallin said that in Paris in 1866 Horteloup and Barth had disastrous results using copper to treat cholera cases. He quoted Bailly as saying that in 1832 and 1849 Bornel remained unharmed by cholera although only 4 kilometers from Chambly, which was almost decimated; at this time, however, copper was not manufactured at Bornel!

Cowley (162) reviewed most of the controversy regarding copper in cholera and quoted a statement by Dr. Chaumery, who had made observations in the little city in Cairo called Khan Khabil, or the Bazaar, in the narrow streets of which all sorts of business was carried on, including work in copper. Chaumery stated that workers in copper lost their lives during the cholera epidemic, from which Cowley deduced that copper had no preventive action whatever against this plague.

Bochefontaine (38) again presented his arguments against Burq's theory of the prophylactic action of copper in connection with the cholera in 1884. In addition to repeating some of the material presented previously, Bochefontaine stated that one of the first, if not the first, victim in Villedieu of the cholera epidemic of 1849 was a maker of utensils, that is, a worker particularly exposed to the action of copper and whose name was not found in the list communicated to the society and whose son, a doctor of medicine, was practicing at that time in his native city.

Burq apparently made another investigation at Villedieu and presented the results at a meeting of the Society of Biology in 1884. A report of this meeting (163) stated that from this investigation it would seem that some of the assertions of Bochefontaine were erroneous and others were incomplete, not proving anything but that the law of prophylaxis by copper has its exigencies as all laws and its limits of resistance.

According to Broca (164), an error made by Riviere in his useful statistics on the cholera epidemic in Paris in 1884 offered to partisans of the prophylactic and curative virtue of copper an argument of fact that was inexact. According to these figures, of the 971 patients entered in the hospitals from November 4 to 22, there were only 2 copper turners against 23 workers in metals other than copper, while in fact there were 5 cases, which were given in detail. From his observations, Broca concluded that the cholera, slight or severe, attacked copper workers, and he even added that workers in other metals had been fewer at the Saint Louis Hospital than those who worked in copper. Moreover, he thought that if there was immunity it was very precarious, since a month of interruption of work destroyed it.

That there may have been some basis for Burq's contention that copper served as a preventive of cholera may be indicated by later discoveries of the role of copper in the cure of certain diseases and the fact that it has been found that copper is stored more abundantly in the liver than in any other part of the body. Apparently Burq and his followers thought that the copper destroyed the cholera germ and in that way prevented the disease. The efficacy of copper in the treatment of cholera, if it has any in this connection, may be explained by investigations on the role of the liver in the pathogenesis of Asiatic cholera reported by Bifulco (165) in 1932. On the basis of experiments on rabbits, Bifulco found that the hepatic gland was of the greatest importance in the pathogenesis of cholera. He observed that, if the vibriones of cholera are injected into the vein that forms part of the portal system, they cause the syndrome peculiar to Asiatic cholera. On the other hand, much larger doses of the cholera vibriones, injected into the marginal vein of the ear, the heart, or the peritoneum, do not give a similar reaction. He said that cholera is manifested when the cholera vibriones, arriving in the intestine, pass into the small branches of the porta and through them to the liver, where they greatly excite the activity of the liver and, returning to the intestine by way of the bile, determine the characteristic syndrome. To further explain their action through the bile and the intestine, in some cases the very poisonous vibriones are able by passing through the suprahepatic vein to invade the organism already weakened by the infection.

Treatment of Tuberculosis

Apparently copper was used early in the treatment of tuberculosis. In 1912 von Linden (166) stated that, long before her experiments, the two Lutons, father and son, had reported good results using copper salts to treat tuberculosis in the eighties of the past century. Von Linden carried out 4 experiments during 1910-12. In the first experiment with 42 animals, 3 were treated with copper chlorid; in the second experiment with 28 animals, 4, in the third with 42 animals, 17, and in the last experiment with 12 animals, 8 were treated with variously modified copper preparations. The best results were obtained when the initial doses were large, 0.005 to 0.025 gm. Cu and continued with smaller doses 0.001 to 0.002 gm. Cu. With all the copper-treated animals, the diffusion of the tuberculosis was more local, less miliary. The impression was that, with the first injection, the disease in the various organs came to a standstill, and when the treatment was continued at proper intervals the foci healed with greater certainty. With the severer cases of infection, 12 injections of 0.01 gm. copper were required to attain a cure. With weaker infection and good weather conditions, 1 injection - 2.5 mg. Cu - was required to bring about cure and sterility in 8 weeks.

A study begun by Corper (117) in 1911 and published in 1914, bearing on the chemotherapy of tuberculosis, included the investigation of various copper salts to determine whether or not copper possessed any bactericidal action toward the tubercle bacillus, whether or not it in any way influenced the progress of the disease in the animal organism, or, in other words, whether it was of therapeutic value and to determine its distribution in the tuberculosis organism. Copper was chosen because it represented the large class of chemical substances, the heavy metals, which had proved of vast importance biologically, had a reputed value in the treatment of diseases due to higher fungi, such as idismycosis, actinomycosis, and sporotrichosis, and had supposedly high fungicidal and bactericidal properties in comparison to its low toxic properties. The author first summarized briefly the work of von Linden, Meissen, Strauss, and Bodmer. He concluded from his own experiments that copper sulfate, copper acetate, and copper amino acid mixture injected in rabbits intramuscularly in doses totaling 12.0 to 42.9 mg. copper during a period of 37 days - the injections begun 11 days after intraocular infection with tubercle bacilli and before visible development of tuberculosis - did not materially affect the progress of the tuberculous process in the eye during the period of

injection or for 269 days after the injections stopped, even though the doses of the various salts bordered on the toxic limit. The same results were obtained for injection on the 11th day after infection and continued for 34 days with a total administration of 6.6 to 22.1 mg. of copper. Various other amounts of copper compounds given for various periods apparently had no appreciable effect on the progress of the disease as indicated by the condition, duration of life, and character of the lesions. Administration of copper in the form of copper amino acids to guinea pigs for 19 days before and 6 days after infection with tubercle bacilli, totaling 5.7 mg. copper, had no appreciable effect on the course of the tuberculosis as indicated by the lesions. Eight of 11 of the guinea pigs treated with copper amino acids died within 49 to 88 days, whereas only 1 control died in 62 days.

In regard to the distribution of copper in the tissues of tuberculous animals Corper found that copper in simple salt form (sulfate, acetate, oleate, and copper amino acid mixture prepared from hydrolyzed egg albumin) injected intramuscularly into normal and tuberculous guinea pigs, in total amounts from 0.75 mg. to about 19.0 mg. for about 2 to 100 days, enters the animal organism and is found mainly and in largest amounts in the liver, in small amounts in the kidneys, in traces in the spleen, lungs, and blood, and not at all in the tuberculous lymph glands and pus. After cessation of injections the copper slowly decreased in amounts in the liver.

Löwenstein (167) in 1924 undertook to test the copper therapy inaugurated by Finckler and von Linden, prompted especially by the favorable reports of Düttmann, who had 22 healed and 3 improved of 29 patients treated with copper; in 1914 Oppenheim had reported the cure in a relatively short time of 13 and the essential improvement of 6 of 19 cases of bone and articular tuberculosis treated with a copper preparation. Löwenstein himself treated 25 cases and stated that although he did not, like Düttmann, obtain surprising results in the majority of cases and in a short time, he did get the impression that the treatment in many cases indicated a certain healing action - with complete lack of effect in the majority. The doses given were 0.2 percent emulsion and 2 percent paste locally and up to 3.5 cc. of a 1-percent solution intravenously at intervals of several days. No injurious effects were observed from this treatment. Four of 25 cases indicated a more or less favorable effect from the copper treatment.

Franke (168) in 1924 reported the treatment of tuberculous fistula with caustic solution consisting of copper and zinc sulfate, 6 to 88 gm. water. The skin was protected from cauterization by rubbing with vaseline or oil. One case of stubborn fistula recovered after the fifth injection. There was a pause of 3 to 4 weeks between the injections.

According to Seabra (169), tuberculosis reduces the copper content of the lung, depending on the extent of the lesions (extent of lung involvement).

MacDowell (170) found that gold and copper had a real therapeutic action when administered to tuberculous subjects. Copper was just as efficacious as gold, with none of the latter's dangers. The copper salt was injected directly into the pulmonary cavities, the doses consisting of 2, 5, 10, and 20 cc. of colloidal copper morrhuate. Sanocrysium or aurothiosulfate of sodium was the gold compound used for comparison.

Mattausch (171) used an oil-soluble copper-chlorophyll compound, activated by small quantities of iron administered in keratin capsules, in treating 218 patients with mild or average chronic pulmonary tuberculosis. Each capsule contained 0.1 mg. copper, 1 mg. iron, and 18 mg. chlorophyll, all constituents being in the

lipoid-soluble form. The daily dosage as a rule was 3 capsules, 1 after each of the principal meals. This was continued for 10 to 12 weeks, interrupted for 8 to 12 weeks, and then resumed again for 2 to 3 months. In some cases a second and third period of medication followed after the usual interruptions. A noticeable change in the reaction of the organism, that is, a reduction of the increased sensitivity and a change toward an increased resistance, was noticeable in approximately 70 percent of the cases after the first few weeks of treatment. This alteration was characterized by decrease or cessation of the toxic processes, by decrease in an eventually existing acceleration of the sedimentation speed of the erythrocytes, by gradual disappearance of the increased metabolic rate, and by increased reserve of the organism when, during the stage of increased irritability, a shifting had taken place toward acidity. The patients felt better, new focal infiltrative processes began to disappear, the erythrocyte and hemoglobin values increased, and the patients gained weight.

Treatment of Cancer

According to Weil (172), interest in the therapeutic effects of colloidal solutions of the metals had been very active for about 15 years before 1913. This interest was largely confined to the effects of these substances in the infectious diseases, owing to the fact that collargol, in particular, had been highly recommended and widely used in gonorrheal ophthalmia and in general sepsis. In Germany and France in 1912 attention was drawn to the fact that experimental tumors in animals were very strikingly influenced and in some cases cured by intravenous injections of colloidal solutions of the heavy metals and their compounds. Loeb and his coworkers had published a preliminary article on the effects of colloidal copper in human cancer, with a detailed report on eight cases. In a summary of their results, quoted by Weil, they stated that they were able to cause gradual retrogression of human cancer, which until that time had withstood various methods of treatment; furthermore, the treatment did not seem to be limited to one kind of cancer but was applicable in the effective treatment of various kinds.

Weil (172), using Loeb's methods, treated a series of patients, but his experience did not entirely coincide with Loeb's statements that, although the earlier injections were followed invariably by a rise of temperature and frequently by a chill, these manifestations decreased in severity with subsequent injections and the general condition (appetite, strength, complexion) improved. Weil did not observe that the general condition improved; on the contrary, his cases, almost without exception, showed a slight though steady loss of weight, as a rule not exceeding 5 to 10 pounds; hemoglobin determinations showed steady deterioration of the blood, ranging from 10 to 20 percent of the original hemoglobin estimate before use of the injections. These undesirable, although by no means serious, sequelae seemed to Weil to be attributable to the effects of the copper rather than to the natural progress of the disease and its associated cachexia (general ill health); many patients improved perceptibly with cessation of treatment. Weil found that the nausea, sometimes associated with vomiting, which occurred occasionally and sometimes interfered materially with proper nutrition of the patient, a more disquieting symptom than those described by Loeb. The absence of copper from the tumors in two cases observed by Weil was considered by him of some importance. In one case the patient had been given almost daily injections of the copper solution for a month, during which period he had received in all 11-1/2 liters. The injections were discontinued a few days before the operation. A mass of tumor tissue weighing a little over 60 gm. was examined chemically for copper without the slightest reaction. In the other case, necropsy was obtained of embryonal sarcoma. The patient had received about 3 liters of copper preparation and was under treatment until the time of his death. No trace of copper could be found in a 30- or 60-gm. portion of tumor tissue. Weil concluded that, judged by certain

clinical criteria, which have been adopted as a reliable standard of therapeutic effectiveness, the treatment had not appeared to exert a destructive action on tumor tissue.

As copper is toxic to the lower forms of living organisms in doses that are nontoxic to human beings, Chatterji (173) thought it could reasonably be presumed that, if the theory of parasitic origin of cancer is accepted, copper should have a parasitocidal effect. Although its method of action has yet to be proved, it is known that the metal acts by forming peroxides analogous to peroxide of hydrogen and peracids, and analysis of human fat and cancer fat shows a proportionate increase in the amount of unsaturated fatty acid in cancer. As Nim oil had been recommended by the Ayurvedic system in treating cancer, Chatterji got the idea of combining the therapeutic properties of copper and the fatty acids derived from Nim in treating cancer. Four cases of malignant tumor were treated successfully by him with copper margosate combined with ethyl ester margosic.

An investigation, mainly preliminary, was made in 1921 by White (174) to determine whether the copper content of tumors differed from that of other tissues and how widely copper was distributed in the organism. Although it has been known for a long time that copper plays an important physiological role in some of the invertebrata, especially molluscs and arthropoda, its presence in the higher animals has usually been regarded as an accident and of no physiological importance, except that it enters into the formation of the pigment turacin of birds' feathers, in which copper exists in combination with hematoporphryn. However, it might have a physiological action as a catalyzer; the presence of an increased amount of copper in degenerated tumors suggested that the degeneration might be associated with an increased catalytic action due to the increased amount of copper, or it might be that copper like calcium tended to be deposited in degenerated areas. Copper was found in over 100 specimens of animal substance examined, except for 1 specimen of urine and 1 sample of egg white, leading to the conclusion that copper is present in tissues to a greater extent than is usually supposed. Twenty-four specimens of tumors examined showed greater variation in copper content, with a minimum of 14.9 and a maximum of 262 mg. per kilogram of dry tumor.

Sugiura and Benedict (quoted by Hieger (175)) had in 1922 tested the effect upon rat carcinoma of 32 salts of a large number of metals (Na, K, Cu, As, Mg, Zn, Hg, Ca, Sr, Ba, Mn, Fe, Co, Mo, W, U, Se, Te, Ge, Pb, Zr, Os) given by mouth and found that copper showed the most definite inhibitory action upon the growth of the tumor. The metal was given as an aqueous solution of CuSO_4 mixed in food in strength up to 0.5 percent. In 1926 Hieger (175) experimented with the administration of copper according to the method used by Sugiura and Benedict to rats bearing inoculated adenocarcinoma. The copper compounds were administered by mouth; 100 cc. of copper salt solution was mixed with 100 gm. biscuit meal. The following copper solutions were used: (a) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 0.3-percent solution (the rats did not appear to be in good health when given 0.4 percent); (b) copper glycine, 0.25 percent; (c) a solution obtained by mixing solutions of NaOH and CuSO_4 in the presence of enough cane sugar to prevent precipitation of cupric hydroxide. The control rats received the same food without the copper. The duration of the experiment was usually 2 to 3 months, depending upon the rate of growth of the tumor. In some series copper was given for 1 or 2 weeks before grafting of the tumor. It was found that a rat bearing a carcinoma and receiving no addition of copper to the food showed 2 to 4 times as much copper in the tumor as in the rest of the body, reckoned in percentage of dry weight. The difference was due perhaps in part to the less cellular tissues (fat, bone) in the body. When the copper compounds mentioned were given to carcinoma-bearing rats with the food for periods of 2 to 3 months, the average amount of copper in the body was not increased by more than 75 percent; large individual variations made it difficult

to obtain exact results on this point; a few normal rats contained more copper than the average amount found in copper-fed rats; the average amount of copper in the tumor could be increased by about 66 percent. The ratio between the amounts of copper in the tumor and in the rest of the body was therefore not distinctly altered by giving copper with the food. In some series of rats the administration of copper seemed to check the growth of carcinoma, but this effect was not obtained constantly.

In a statement on the use of colloidal lead in the treatment of cancer, Wood (176), briefly summarized some of the work on copper as follows:

The use of colloidal metals in the treatment of malignant diseases was first recommended by Torres in 1904. Neuberg tested a number of colloidal metals. Subcutaneous injections showed no effect; but after intravenous injections of various colloidal metals, including lead and copper, changes were noticed in the mouse tumors treated, especially with cobalt and silver. Various copper preparations were tried by Loeb and Gaube, especially cuprase, which is a colloidal copper hydrate. Loeb obtained no permanent results. Gaube noticed that considerable improvement in human beings took place, but his observations were over too short a period to permit of any final conclusions. The matter was thoroughly studied on human beings by Richard Weil, who concluded that as much damage was done by the colloidal copper as was benefit received. Phlebitis and periphlebitis were observed; also hemoglobinuria and renal lesions. The liver was not much injured, though it contained considerable copper. Curiously enough, practically no copper was found in the tumor.

Pirrie (177) has reported that iron and copper are markedly changed in their concentrations in the blood serum of cancer patients compared with the normal concentration in the serum of healthy people.

Pirrie measured the copper in the serum of 40 healthy and 19 cancer-stricken people and found that, in those with cancer, the copper content averaged over twice as high as normal, but the iron was well below normal. This was true for all types of malignancies, those observed being as diversified as, among others, cancers of the lung, bone, and breast.

Treatment of Anemia

McElroy and Swanson (178) mention the fact that everyone knows how important to health vitamins are, but less well known is the vital role of the so-called trace elements (micronutrients), a group of metals needed in infinitesimally small amounts but without which neither animals nor plants can thrive. Of the 60 elements that have been found in the cells of living things, only 8 are considered important. These are iron, copper, zinc, manganese, cobalt, iodine, boron, and molybdenum. The first 6 are essential to animals, while the first 4 and the last 2 are necessary to plants.

A deficiency of iron produces anemia in animals. Cobalt and copper also are in some way tied up with the formation of hemoglobin, for anemic patients who do not respond to iron dosage often do when cobalt or copper or both are added to the diet. Cobalt apparently is involved in forming the newly discovered antipernicious anemia vitamin B₁₂. Apart from maintaining the hemoglobin content of the blood, copper also is an important part of hemocyanin, the respiratory pigment of invertebrates. A disease of Australian sheep that has been traced to a lack of copper produces marked and permanent effects on the nervous system. The first sign

of this form of anemia is deterioration of the wool. The individual strands lose their kinkiness and strength and take on a lustrous sheen. It is easy to detect this early symptom, and copper can be supplied before the damage becomes permanent (178).

In 1928 in studying the factors operating in the cure and prevention of anemia produced in rats by a diet of cow's whole milk, Waddell, Elvehjem, Steenbock, and Hart (179) found that inorganic iron salts (chloride, sulfate, acetate, nitrate, and phosphate), when fed at such a level that they introduced 0.5 mg. of Fe daily (6 times per week), failed to increase materially the hemoglobin level in anemic rats; the ashed residues from dried beef liver, dried lettuce, and yellow corn, and acid extracts of the same, when fed at such a level that they introduced 0.5 mg. of Fe 6 times per week, were very effective in curing the anemia. These results were explained by assuming that the ashes and ash extracts contained, in addition to iron, some other inorganic substance or substances vitally concerned in the building of hemoglobin.

While in the midst of investigations previously reported, Hart and coworkers (180) obtained for experimental purposes, before it appeared on the open market, a generous quantity of a liver preparation. It was desired to find out whether this would be effective in treating anemia in view of the fact that it was low in iron. The hemoglobin levels of the animals receiving only the preparation remained low, while those animals receiving iron in addition to the preparation increased their hemoglobin very rapidly. The ash of the preparation and extracts of the ash, when supplemented with additional iron, were very effective in curing anemia in the rats. These results suggested that the preparation might represent a fraction containing in greater concentration that substance (or substances) that the authors had assumed as being present in the ashes of yellow corn, dried lettuce, and dried beef liver. Shortly before these fractions of the preparation were studied, a trial was made of the effect of adding 0.25 mg. daily of copper as copper sulfate plus 0.5 mg. of Fe as ferric chloride added to the whole milk diet. This trial of a copper salt was made because a test was being carried out by these investigators on all inorganic elements recognized as present in biological materials as supplements to iron, and the immediate use of copper was suggested by the fact that a pale blue color had often been noticed in the ash of some of the materials studied, particularly the ashes of lettuce and the commercial preparation. Copper was also suggested by the knowledge that in some of the molluscs and crustacea it is known to exist as an integral part of the compound hemocyanin, which functions as a respiratory pigment similar to hemoglobin in the higher animals. The response to this preliminary experiment with copper, which according to the authors was the first in the literature giving copper in association with iron for the specific function of hemoglobin building in a mammal on an otherwise satisfactory diet, was surprising. It was considered as more than a casual incidental connection that the commercial preparation was effective in treating anemia in rats exactly as it was in the treatment of pernicious anemia in man and that the effectiveness of this preparation could be duplicated in experimental animals by using copper alone. The presence of copper in the extracts was considered presumptive evidence that the copper was responsible for the effect produced.

Later in 1928, Robscheit-Robbins, Elden, Sperry, and Whipple (181) published an article in which they stated that about 8 years before Robscheit-Robbins and Whipple had observed that feeding apricots had a potent influence in experimental anemia in dogs. These observations were not published, because the reaction was so unexpected as to suggest the possibility of error. A few years later these observations were repeated and extended to show that apricots and peaches (fresh, dried, or canned) were quite potent in severe anemia due to hemorrhage in dogs. The addition of 200 gm. of this cooked fruit to the daily standard diet caused an average output of 40 to 45 gm. of hemoglobin per 2-week period over and above the control periods. All

the evidence pointed to salts as perhaps responsible for at least a part of this unexpected reaction. Some crude combustion products containing much carbon were then prepared and found to be potent in this experimental anemia. Finally, more complete combustion of the dried apricots was carried out, and this pure inorganic material was likewise found to be potent, suggesting that some of the metals were concerned in the reaction. Logically the potent organ tissues were next tested by ashing and feeding in the experimental anemia with almost identical results. The results did not wholly accord with the observations of the Wisconsin investigators (Hart and his coworkers), but the divergent results could be explained by differences in the anemia or in the experimental animal. The Wisconsin investigators studied a nutritional anemia, whereas Robscheit-Robbins and associates used a simple anemia due to hemorrhage in otherwise normal dogs. On the whole, the latter found the reactions quite uniform, which indicated that, in the hemorrhage type of anemia, the inorganic ash of various food products contained salts of evident potency that influenced favorably the regeneration of new red blood cells and hemoglobin in their standard anemic dogs.

Robscheit-Robbins and associates concluded that feeding apricots was surprisingly potent in standard anemic dogs and caused a large output of hemoglobin above control levels, which might amount to 40 to 45 gm. of hemoglobin per 2-week period. The inorganic ash of dried apricots retained most of the potency of whole apricots and exerted the same favorable influence upon the red cell and hemoglobin regeneration when incorporated into the diet of standard anemic dogs. The inorganic ash of beef liver and pig kidney also was found to be potent in this type of experimental anemia, but the ash contained only about half the potent factors present in whole cooked liver or kidney. The liver or kidney ash added to the diet might increase the hemoglobin output by 40 to 70 gm. per 2-week period.

In a later paper Robscheit-Robbins and coworkers (182) stated that the influence of copper tartrate or sulfate was slight when added to the standard diet in their experiments and much less than iron salts or the ash of apricots, liver, or kidney. They concluded that -

Certain metals and their salts when added to the standard diet of anemic dogs have a distinct influence and increase hemoglobin production. Copper has a positive effect, although moderate in degree, and usually increases somewhat the hemoglobin production above control levels. The iron salt effect is much more notable than the copper effect. Combined copper and iron feeding may at times have an unusually favorable effect or again may not exceed the favorable influence of the iron salt feeding alone. At this time it would be well to keep in mind the experiments of Mallory and his coworkers which indicate that copper salts may be toxic. The doses used by us are large and we note that some animals are much more susceptible than others. It is not known whether humans are more sensitive to copper than animals nor is it known whether an anemic patient may be more sensitive than a normal person. This point should be emphasized to indicate reasons for caution in the administration of copper salts in human anemia, as a toxic cirrhosis would be more of a liability to a patient than even a stubborn anemia which might yield to suitable diet therapy.

The attention of Titus, Cave, and Hughes (183) was directed toward a study of manganese when rabbits, which had been fed for some time a corrective diet containing Fe_2O_3 of "analyzed" purity, continued to grow and failed to become anemic as expected. Manganese added to a milk-iron diet seemed to give almost if not quite as good results in the building of hemoglobin as did copper added in the

same way. Manganese and copper added to a milk-iron diet appeared to produce a quicker response from the standpoint of hemoglobin building than did either copper or manganese when fed alone as a supplement. The experimental data presented seemed to be in line with Whipple's suggestion that "the evidence points to a group of active substances rather than to a single active principle."

To ascertain the extent to which minerals may act as catalyzers and replace the so-called "vitamins", Chidester and Eaton (184) made experiments with a number of chemicals added in minute doses to the food of experimental rats showing unmistakable signs of avitaminosis. As experiments with iron and iodine proved successful, it was decided to try copper in the presence of iron and arsenic. In experiments with 15 negative-control and 8 positive-control rats, 6 other animals were placed on a diet that included ferrous sulfate, iron iodide, arsenic, and copper sulfate added to the Sherman A-free diet No. 380. In the first experiment, 2 male animals received daily 1 drop of a solution of copper sulfate, adding 0.01 mg. of copper to their food. They received 1 drop of ferrous sulfate solution containing 0.5 mg. of iron and 0.01 mg. of ergosterol, which had been irradiated with a small quantity of the Sherman diet No. 380. They also received in the drinking water 1 cc. of dilute Fowler's solution of arsenic containing 0.379 mg. of arsenic. Beginning with an average weight of 42 gm. the rats reached a maximum weight of 175 gm. at the end of 9 weeks. After fluctuating around 170 gm. for 2 weeks a rapid decline resulted in death at the end of 13 weeks. Autopsy showed that in one case the left ear and left adrenal were infected, while the other specimen had infected kidneys, testis, and gall bladder. Experiments with 4 females receiving Sherman diet No. 380 and irradiated ergosterol, 1 drop of ferrous sulfate solution, and 5 drops of dilute syrup of the iodide of iron were also unsatisfactory. Averaging 53 gm. at the beginning of the experiment, the animals reached a maximum of 160 gm. at the end of 12 weeks; for 7 weeks they fluctuated around 157 gm. The results indicated storage but no benefit. The results obtained differed from the effects observed by Hart with 0.01 mg. of copper sulfate given daily to rats, in addition to 0.5 mg. of iron in curing anemia, as the results obtained by Chidester and Eaton did not persist. They hoped, however, to find the optimum minute amount of copper that would be effective in combination with the other minerals they had determined as catalyzers of value in avitaminosis.

In another article from the University of Wisconsin Laboratory, published in 1929, Waddell, Steenbock, and Hart (185) stated that

In 1928 it was reported from this laboratory that inorganic iron salts when added to a basal diet of whole milk at a level of 0.5 mg. of Fe daily (6 days per week) were ineffective in curing a nutritional anemia in rats. It was also observed at that time, although not reported, that the same iron salts fed at a level of 2 mg. of Fe daily stimulated hemoglobin production. These iron salts had all been prepared from the same standard iron wire with pure reagents. It was apparent that either the increased intake of iron was causing this increased hemoglobin production or that there was present in the iron solutions some contaminant which brought about a greater response. Therefore, it was decided to investigate the hematopoietic action of even higher doses of iron, especially purified with regard to copper. A pure iron salt, ferric chloride, when fed at very high levels did not serve to correct the anemia induced in young rats by a milk diet. These experiments serve again to emphasize the importance of very small amounts of certain elements in the animal organism, and especially the importance of copper for hemoglobin formation. The so-called pure iron wire used in the previous investigation which showed regeneration from high

doses of iron contained sufficient copper to affect markedly the hematopoietic function in rats, and copper has been found by analysis to be a contaminant of many iron salts. These results may serve in some measure to explain the many and conflicting reports in the literature with regard to the effect of iron in curing or preventing various experimental and clinical anemias. The results of Beard and Myers might be explained in this way.

Another experiment was carried out by Waddell, Steenbock, and Hart (186) to determine whether other active elements in addition to copper were present in the fraction precipitated in a previous experiment (179) by hydrogen sulfide from an acid extract of the ash of the commercial liver preparation. They undertook to separate the copper from the other elements present by electrolytic deposition of the copper. The electrolyzed copper and the residue from the electrolysis of the hydrogen sulfide fraction were then fed to anemic rats, being added to a basal diet of whole milk and iron. Besides these substances, 0.05 mg. of copper was also fed as a solution of pure copper sulfate. Both the copper electrolyzed from the hydrogen sulfide fraction and that from copper sulfate brought about prompt and marked increase in the hemoglobin level and body weight, while the residue remaining from the copper electrolytes was quite inactive. These results showed convincingly that the only material present in the extract of the ash of the liver preparation put out by a commercial company was copper and that copper obtained from a pure copper salt was equally efficacious as that electrolyzed from the extract of the ash. This was considered to be additional and convincing proof that the deficiency of the basal diet was inorganic in nature and that the inorganic deficiency was copper only.

As copper apparently is not a constituent of the hemoglobin molecule and as, therefore, it increases the hemoglobin formation in some indirect way, the question arose as to its specificity in this reaction. Waddell and his associates (187), immediately upon discovering this role of copper in nutrition, tried to determine whether any other element had the property of supplementing iron in curing the anemia produced by a diet of whole milk. In paper IX (187) of this series, data were presented showing that copper was the only element present in a variety of liver preparations that had the ability to supplement a basal diet of milk and iron. The fact that the supplementing effect of various liver preparations was in direct proportion to their copper content offered strong presumptive evidence that this element, among those normally present in the animal body, was entirely specific in its antianemic action. The experiments described in paper X were carried out with 12 elements - zinc, chromium, germanium, nickel, lead, cobalt, antimony, cadmium, tin, mercury, arsenic, and manganese. Experiments were also carried out with a mixture of the 12 elements studied. According to the investigators (186), the results of these experiments showed clearly that none of the 12 elements can replace copper in curing the anemia produced by the basal diet. All of them (with the possible exception of arsenic) are distinctly negative in their effect, and arsenic brings about only temporary and minimum response in the production of hemoglobin. It was concluded that copper, therefore, is quite unique in its action and must be considered a necessary element in animal nutrition.

Waddell and coworkers (186) call attention to the investigations of Whipple, Robscheit-Robbins, and their associates, who emphasized the fact that the anemia produced by them is different from the simple deficiency anemia produced by the milk diet. They bring about a deficiency by excessive hemorrhage, which is undoubtedly multiple in nature. The use of a diet, which is admittedly complete for normal life processes, together with the loss of many substances to the body by hemorrhage, seems too complicated for elucidation of the role of any one substance in hematopoiesis. This would seem to apply particularly if the substance in question

were necessary only in small amounts. It is to be expected (186) that the evidence would point to a "group of substances" rather than to one substance as being responsible for the increased formation of hemoglobin. Whipple and his associates reported that, although iron and copper had a stimulating action, iron alone was more effective than copper alone, to which Waddell replied that need for iron is undoubtedly greater than need for copper, as it is a constituent of the hemoglobin molecule and is lost to the animal body by bleeding. In view of the fact that Whipple's basal diet undoubtedly contained some copper, Waddell thinks it is not surprising that iron would have a pronounced effect. Waddell suggests that the "salt effect" or "catalytic action" of high iron dosage observed by Whipple may have been due to small amounts of copper contained in the iron, as in studies with rats high iron intake did not increase hemoglobin production above that obtained with smaller doses when the iron was carefully freed from its copper contaminant.

Elvehjem and Hart (188) found that day-old chicks placed on a diet of cow's milk, polished rice, calcium carbonate, and sodium chloride invariably became anemic. The hemoglobin fell from 8 gm. per 100 cc. of blood to 4 gm. per 100 cc. in 12 to 15 days. Additions of ferric oxide to this ration did not prevent the anemia, because the iron in ferric oxide is not assimilated by chicks. The addition of ferrous sulfate or purified (copper-free) ferric chloride to this ration immediately stimulated hemoglobin synthesis because this basal ration contains small amounts of copper. When purified ferric chloride was added to a modified basal ration very low in copper, no stimulation was noted until minute amounts of copper were added.

The results of the experiments with chicks verify again the great anemia-producing power, that is, the low iron and copper content, of whole milk. No difference was found in the animal used. However, in an animal like the chick, which will not grow normally on a milk diet alone, the introduction of additional food, unless highly purified, has a favorable effect upon hemoglobin synthesis. These results, as well as actual analyses of these foods for iron and copper, demonstrate the universal distribution of these hemoglobin-building elements in most natural foods. This fact is considered of great importance, whether one is interested in producing anemia in experimental animals on any diet beside milk or in the use of natural foods as a supplement to milk to prevent anemia in young growing animals. Great care must be taken in preparing materials other than milk to produce anemia in experimental animals. Several natural foods may be purified to such an extent that they are low enough in iron to produce anemia, but it is very difficult to reduce the copper content enough to maintain the anemia when enough iron is added to the diet.

Elvehjem and Hart (188) call attention to a report by Drabkin and Waggoner which states that the severe anemia developed in rats on a milk diet could be cured by placing these rats on a copper-free synthetic ration. However, analysis of this copper-free synthetic ration showed that it contained 0.044 mg. copper and 0.532 mg. iron per 10 gm. of the ration. Assuming that the rats consumed 10 gm. each of the ration per day, they would be ingesting the optimum amounts of iron and copper for hemoglobin regeneration. The importance of careful estimations of copper in anemia-producing diets cannot be overemphasized.

Whitehead and Barlow (189) induced a secondary anemia in rats by feeding a diet consisting of polished (cooked) rice and salts. With such a dietary, the body weight and hemoglobin values were diminished to a greater extent than the red-cell counts. The addition of 2 percent compressed yeast to polished rice formed a virtually balanced diet for rats. The erythrocyte counts and hemoglobin levels were maintained within a normal range, and the body-weight losses were much less than with rice alone. Yeast, however, was less active as a curative than as a prophylactic measure in rice disease. Additions of vitamins A and D in the form of cod-liver oil were

ineffective in preventing the onset of polished-rice anemia. Therapeutic doses of iron and copper either singly or in combination were ineffective either as curative or prophylactic measures in rice anemia. Lean beef muscle or liver either added to the rice diet or substituted completely for the cooked rice resulted in a more rapid and complete remission of the anemia of rice disease than was observed with any other procedure tested.

Rats made anemic by whole milk diet, according to Smith (190), were not benefited by adding inorganic iron (ferric oxide); but cabbage, fresh alcoholic extracts of dried cabbage, or yellow corn added to the milk immediately cleared up the anemia. It was suggested that "copper acts as a catalyzer for some reactions concerned in hemoglobin building just as iron functions in the production of chlorophyll although it is not a constituent of the chlorophyll molecule." Hemoglobin production after experimental hemorrhage was not affected by chlorophyll, but smooth and striated muscle helped materially. Gizzard, the purest form of smooth muscle tested, gave results superior to other materials. Surprisingly good results were obtained with cooked dried apricots, peaches, and prunes. These ranked in the same class with certain meat products. Raisins, fresh grapes, and apples fresh and dried were less effective, and fresh black raspberries were inert. Copper salts tested alone or with zinc produced slight or no results, but when iron was added its effect was accelerated, that is, more hemoglobin was produced.

In a preliminary report on the beneficial effect of combining small quantities of copper salt with iron to treat idiopathic anemia of the hypochromic type, Mills (191) stated that -

So far as can be ascertained from the literature, copper and iron mixtures have never been prescribed for the treatment of this disease, although their effects on experimental anemia in animals have been demonstrated. The effect of other inorganic substances than copper added to iron for treating experimental anemia has been found to be slight or nil.

The four cases given here are representative of a series of 10 cases of idiopathic hypochromic anemia treated by means of an iron-copper mixture. They are not "picked" cases. All remaining cases showed similar results and promised to compare favorably with those reported.

The copper mixture - Bland's mass 2 g., copper sulfate 1.5 mg., and a laxative - was given in capsule form. It is of interest that several of the cases showed distinct though temporary improvement when taking Bland's capsules along with a diet containing calf's liver. In the light of the results obtained from Bland's and copper it would seem that the beneficial effect of the liver-Bland combination was due to the copper content of the liver which is considerable, according to a recent analysis of 145 mg. per kilo of fresh liver. Any explanation as to why copper is of such value when combined with iron in the treatment of these cases is largely speculation. It may be a catalyst as Waddell and his coworkers suggest. Certain of its salts are known to be good catalysts in vitro and recent observations on the oxygen-combining power of dog's blood after heavy copper feeding suggest that it may have this same catalytic action in the circulating blood.

In an article on manganese, copper, and iron content of serving portions of common foods, Hodges and Peterson (192) state that:

The last 5 years have witnessed the development of a marked interest in the distribution of certain mineral elements that occur in only small quantities in our foodstuffs and a rather definite belief that several of these elements play a much more important role in nutrition than has hitherto been assigned to them. This widespread interest is evidenced by an increasing number of papers dealing with the analysis of our common foods and by numerous investigators to determine the function of these elements in the animal body. The French investigator, Bertrand, has been a conspicuous pioneer in focusing public attention on such elements as manganese, zinc, cobalt, and nickel. McHargue, in this country, has published many papers during the last decade regarding the distribution of these minor elements, and has made numerous suggestions regarding their importance in biological processes. Public attention was sharply focused on one of these elements by the brilliant discovery of Hart, Steenbock, and associates that copper possesses the property of supplementing iron in hemoglobin formation in anemic rats. These investigators demonstrated that nutritional anemia in other animals, namely, chickens and pigs, could be corrected by the addition of copper to the ration. While some investigators do not agree with the Wisconsin group as to the specificity of copper for hemoglobin formation, the consensus of opinion is that copper occupies a unique and important position in this respect. Mills has recently published a paper showing the remarkable benefits derived from ingestion of copper by humans in cases of idiopathic anemia.

In view of the recognized importance of manganese, copper, and iron in nutrition it becomes a matter of some consequence to know the copper and manganese content of our common foods and desirable to have the mineral content expressed on a more readily usable basis than the customary fresh or dried basis. * * * * *

The manganese, copper, and iron content of any diet depends on the amount and kind of food eaten. In the different menus taken from Rose's Feeding the Family, copper varied from 0.63 mg. for the 3- to 4-year old child to 4.8 mg. for the working man. The maximum figure is due to the high caloric intake, i.e., quantity of food in the menu. The average copper intake of all the family menus was 2.26 mg. The intake of copper was lower in summer, being 1.91 mg. compared with 2.60 mg. in winter. The food portions richest in copper are calf liver, oysters, beef liver, mushrooms, currants, and chocolate. Cereals supply the largest proportion of manganese and copper in these diets. Vegetables and cereals are the chief contributors of iron. Fruits are an important source of all three elements.

According to Mackay and Goodfellow (193), review of the literature of anemia in infancy reveals that there is no general agreement concerning the cause, course, or treatment of anemia in premature infants, or as to whether or not this anemia is a separate clinical entity from the anemia occurring in full-term infants. Some have held that the so-called simple anemia in full-term infants is analogous to chlorosis in the adult, others that it is due to a toxic effect of cow's milk, others again that it is secondary to a great variety of factors, especially dietetic errors and microbial infections. Many believe that it may be caused by iron deficiency, but it has usually been stated that the period of milk-feeding must be unduly prolonged to cause the appearance of anemia in full-term babies. In the most comprehensive recent book on the blood diseases of childhood, anemia due to iron

deficiency is held to be rare, and Mackay and Goodfellow hope that the investigations described may help to render it rare by establishing an opposite view. In referring to the work of Waddell and associates, they state that copper, being a usual constituent in iron salts, has no doubt played a part in the good results previously reported from iron medication and may account for the superiority of large doses of iron over small doses.

From their own experiments, Mackay and Goodfellow (193) concluded that treating infants with iron and ammonium citrate prevented and cured anemia, raised the resistance to infection, thereby greatly reducing the morbidity rate, and considerably improved the rate of growth. Curative treatment should follow the same lines as prophylaxis. Doses of iron and ammonium citrate, where possible, may be pushed higher, especially in older infants, but it is essential to success to begin treatment gradually. The effect of increasing the amount of copper given with the iron is not yet known. In view of the results obtainable by Mackay and Goodfellow, they think it is difficult to understand why so many authors have stated that iron is ineffectual, though the following factors may afford an explanation of certain failures: Too short a period of treatment; presence of pyogenic infections or disturbance of the alimentary tract; and use of iron salts of low solubility or of absolute chemical purity.

Funk and Lejwa (194) studied the food factors affecting red cell formation. Fractions of liver and yeast extracts fractionated by the use of lead acetate, alcohol, picric acid, and the picrates of silver and mercury, and also a copper and iron combination, were injected into rats at intervals of 2 days, and their effect on the red cell count over a 12-day period was determined. Copper and iron produced a diminution, but the diminution produced by total extracts of both yeast and liver was still more marked. They suggested that the anemia produced in rats by a milk diet and cured by a supplement of copper is a "physiological" anemia. When the copper deficiency is supplied, the increase in red cells is not due to the specific stimulus of copper but to the fact that the rats survive to the age when there is a normal increase in the red cells.

Witts (195) treated chronic mycrocytic anemia successfully with large doses of iron by mouth, emphasizing that large doses are absolutely necessary. He stated that copper plays no part in the action of the large doses of iron. An analysis of the preparations of iron used revealed between 3 and 4 mg. of copper per kilogram, which would give only 0.01 mg. of copper per day, or less than the food contained.

Young (196) used $1/8$ gm. of copper in a pint of water daily for one patient with success and thought that injections of colloidal copper were of some value in the secondary anemias of malignant disease.

Keil and Nelson (197) refer to the fact that the relation of copper to hemoglobin formation has attracted the attention of a number of investigators during the past few years. Hart and his coworkers were the first to point out that copper plays a specific role in building hemoglobin and that milk fortified with pure iron salts does not constitute an adequate diet for regeneration of the respiratory pigment. Eyvard, Nelson, and Sewell showed that rats and swine make better gains and exhibit a higher food utilization per unit of weight increase when small amounts of copper sulfate are incorporated in the ration. In the ashing of the rats fed copper sulfate, it was observed that the greater part of the stored copper was confined to the liver, which might be the reason for the medicinal and nutritive value of liver. Myers and Beard found, however, that iron alone was sufficient for regeneration of hemoglobin and that other elements besides copper could increase the speed of regeneration, particularly manganese, nickel, germanium, and arsenic. Keil and Nelson (197) studied vanadium, titanium, manganese, copper, nickel, arsenic, germanium, zinc, chromium, cobalt, tin, and mercury. They found that none of these

substances, except copper, caused regeneration of hemoglobin. Pure iron as ferric chloride, when added to milk collected in glass, did not cause regeneration of hemoglobin. Copper was the only element that had a positive effect on hemoglobin building. Nutritional anemia produced a distinct change in color of the dark-coated rats, and addition of pure iron as ferric chloride did not restore the color; however, addition of 0.05 mg. of copper per day to the whole milk and iron as ferric chloride restored the original coat in about 2 months.

The favorable response of 10 cases of idiopathic hypochromemia to fairly large doses of iron and small quantities of copper salt was reported by Mills (198) in a preliminary communication published in 1930. In 1931 Mills (198) presented 23 cases as evidence for considering idiopathic hypochromic anemia or hypochromemia (low color index of blood) as a disease entity. The important clinical features are absence or great diminution of free hydrochloric acid in the gastric contents, absence of etiologic factors, a hypochromic blood picture, and a tendency to resist ordinary forms of anemia therapy. No hydrochloric acid was given in the 10 cases reported before. The treatment was suggested by the experimental observations of Hart and others on milk anemia in white rats and later by Whipple's group studying anemia in dogs. The iron preparation chosen was Bland's mass. To it was added copper carbonate in the proportion of 1/48 gm. to 30 gm. of Bland's mass. These amounts were incorporated in a soluble capsule with a small amount of a suitable laxative. The dose was 1 capsule 3 times a day. Larger quantities of copper salts, prescribed in 5 cases in amounts up to 6 gm. daily, resulted in a mild degree of gastroenteritis characterized by anorexia, abdominal cramps, and diarrhea. For control purposes, most of the original cases were placed on a preliminary period of treatment by Bland's mass alone. Sixty grains were given daily. This treatment was continued for 4 weeks before the iron and copper trial. In 5 cases the control period was much longer than 4 weeks. No hydrochloric acid was given unless the cases proved refractory to the iron and copper mixture. In most instances there was only a slight rise or no improvement in the hemoglobin level on the Bland's mass, whereas a decided increase in the hemoglobin promptly followed addition of copper to the mixture. Not the least striking was the improvement in the patients' color. The appetite returned and with it a sense of well-being and a gain in weight. Mills stated that, when this article was written, the original cases had been followed for 1-1/2 years, since the beginning of the therapy, and all cases showing improvement had maintained this improvement with two exceptions. However, there appeared a tendency for the hemoglobin level to decline unless therapy was repeated at frequent intervals. Two cases were failures.

Mills (198) quotes Waugh as confirming the superiority of iron and copper therapy over iron alone in the treatment of this disease, whereas Giffin and Watkins failed to get results with the independent administration of copper and manganese in such cases. This failure would tend to support the contention that it is the iron that is the necessary hemoglobin-building substance, but that it is rendered more available by the action of the combined copper. According to Mills, no explanation of the mode of action of copper has been offered, although it was originally suggested by Hart and others that the copper acted as a catalyst improving the absorption of the iron from the intestinal tract. Whether this is so or whether the copper is absorbed along with the iron and serves in the synthesis of hemoglobin is a problem not likely to be entirely elucidated until more light is brought to bear upon the nature of the hemoglobin molecule.

The activity of copper was measured by Elvehjem and Sherman (199) by comparing the increase in hemoglobin content of the blood of anemic rats when fed pure iron alone with the improvement obtained when iron was accompanied by traces of copper. Special emphasis was placed on the action of copper on storing and utilizing iron in the animal body. When pure iron was fed to anemic rats for 2 weeks, there was no

increase in the amount of hemoglobin in the blood, but there was a decided increase in the total iron content of the liver and spleen. The total iron in the liver increased from an average of 0.12 to 0.46 mg. of Fe and that in the spleen from 0.024 to 0.068 mg. of Fe. When the iron was taken away and 0.05 mg. of copper substituted, the average increase in hemoglobin for the 2 weeks was 2 gm., compared with 0.1 gm. when iron was fed. Most of the increase in the hemoglobin took place during the first week of copper feeding, and in many cases the hemoglobin regeneration had reached a maximum at 1 week and decreased thereafter owing to lack of iron. Copper is unnecessary, according to Elvehjem and Sherman, for iron assimilation but is necessary for the synthesis of hemoglobin. The rate of hemoglobin formation depended upon the iron intake, and the liver showed most iron storage after 0.3 mg. or more of iron was fed.

Elvehjem and Hart (200) call attention to an earlier paper in which they had reported that anemic pigs fed ferric chloride made almost as rapid recovery as those fed both iron and copper. Two possible reasons were suggested for these results: First, the iron reserves in the pig at birth may be depleted before the copper store and as soon as iron is supplied hemoglobin synthesis can take place without copper feeding; secondly, the pigs were not under as restricted conditions as were the smaller animals and probably had access to small amounts of copper. After publication of these results, several workers misinterpreted the conclusions and referred to the work as proof of the fact that not all mammals require copper for hemoglobin formation. Elvehjem and Hart (200) give results obtained when iron and copper were supplied to pigs kept under carefully controlled conditions and fed a milk diet that demonstrated that copper is equally as necessary for hemoglobin synthesis in pigs as in other experimental animals, such as rats, mice, and chickens. From their experience in the laboratory and from all the results reported in the literature, these authors believe they are justified in concluding that, when hemoglobin regeneration takes place with the addition of iron alone, the animal either contains or is receiving enough copper for this purpose from some other source.

Previous procedure had been to study the curative effect of metals on nutritional anemia, whereas the purpose of work carried out by Orten, Underhill, and Lewis (201) was to study the preventive action of certain metals, including those that had been found ineffective in correcting nutritional anemia, and to determine whether the failure of these metals to cause an increase of hemoglobin in anemic animals might have been due to some toxic effect resulting from the comparatively high levels at which the metals were fed. Five groups of animals were studied, with respective supplements, as follows: Group I (7 rats), 0.5 mg. of iron; group II (6 rats), 0.5 mg. of iron and 0.025 mg. of copper; group III (8 rats), 0.5 mg. of iron and 1.0 or 0.1 mg. of manganese; group IV (7 rats), 0.5 mg. of iron with a mixture composed of 1.0 mg. of manganese, 0.5 mg. of cobalt, 1.0 mg. of nickel, and 0.5 mg. of zinc; and group V (6 rats), 0.5 mg. of iron, the same mixture of metals as group IV, and 0.025 mg. of copper. The results confirmed previous observation that iron alone is ineffective in preventing nutritional anemia but in no way agree with the report of Beard, Rafferty, and Myers that inorganic iron has a prophylactic action in this type of anemia. In contrast to the ineffectiveness of iron alone, copper supplementing iron prevented development of the anemia. The animals grew well and maintained an approximately normal hemoglobin level, showing that copper as a supplement to iron is just as effective in preventing as in correcting the anemia. According to Orten, Underhill, and Lewis, these results agree with the findings of Waddell, Steenbock, and Hart. Although manganese was ineffective in preventing nutritional anemia, some beneficial effect was observed on growth and on the length of life.

Referring to the study reported above (201), in which animals receiving a mixture of manganese, cobalt, nickel, and zinc with iron developed a progressive anemia and

died whereas those receiving the same mixture with iron and copper developed an abnormally high hemoglobin value at 7 to 9 weeks, Orten, Underhill, Mugrage, and Lewis (202) reported another study to determine which one or combination of these metals was responsible for this marked increase in hemoglobin and in red blood cells. All rats fed a milk-iron diet supplemented by cobalt without copper, using either a curative or preventive procedure, developed an anemia and died, whereas those given the milk-iron diet supplemented by cobalt with copper developed a polycythemia. It is considered that the results add to the evidence that copper is an essential factor in hemoglobin and erythrocyte production and, furthermore, lead to the conclusion that copper in the diet is necessary for the development of a cobalt polycythemia. Consequently Myers, Beard, and Barnes, and Waltner and Waltner probably were successful in producing a polycythemia with cobalt only because the stock diets used by them contained enough copper to permit the development of the polycythemia. The mechanism involved in producing polycythemia when cobalt is administered is not known.

Josephs (203) carried out experiments on three series of rats as follows:

Series 1, 3 groups of rats. In the first group 35 rats received milk alone after they were weaned; in the second group 19 rats were given milk with an addition of 0.5 mg. of iron per day after they were weaned; in the third group 9 rats received milk with the same addition of iron and 0.1 mg. of copper per day. In rats receiving the addition of iron or iron and copper the retention of iron averaged about 10 percent of the intake; the copper had no influence on the retention of the iron. The iron content of the tissues of the rats receiving copper in addition to iron was, within experimental error, the same as that of the tissues of rats receiving milk alone, which means that virtually 100 percent of the iron retention was used for hemoglobin under the influence of copper. (As the cages were of galvanized wire the animals may have ingested minute amounts of metals not added to the food, so that these experiments cannot be used to demonstrate that copper is not necessary for hemoglobin formation.) After about the thirtieth day of life the concentration of tissue iron reached a level below which it did not tend to go. The animals on milk alone in general failed to grow beyond this point. The tissue iron was maintained at the necessary level first at the expense of the hemoglobin; when the hemoglobin concentration was reduced to its lowest point, the tissue iron was maintained at the expense of the growth of the animal. On the other hand, when iron was plentiful, 80 percent of it went into hemoglobin and this was raised to 100 percent under the influence of copper.

In series 2, litters of rats were fed on milk alone for a period varying from 5 to 58 days; then pairs from the same litter were selected on the basis of similarity of behavior and to the diet of 1 pair was added iron, to the other iron and copper. The amount of iron given varied between 0.5 and 1 mg., and retention varied for the most part between 10 and 20 percent. Copper did not affect the retention to a demonstrable degree. The greater utilization of the retained iron for hemoglobin formation and the consequent smaller deposition of iron in the tissues under the influence of copper were striking.

In series 3, 4 groups of rats were fed as follows: Group 1, 16 rats on milk alone from the time they were weaned; group 2, 8 rats on milk, with the addition of copper; groups 3 and 4, 8 rats on milk alone for a preliminary period, then iron was added to the diet of 3 and iron and copper to the diet of 4 of the rats. Analyses of liver, spleen, and muscle showed that the liver was the only tissue that increased in iron concentration after the ingestion of iron. As a result of the work reported, Josephs divided the tissue iron into two parts - a variable or mobile portion and a fixed portion. The fixed portion did not tend to fall much below a concentration of 2 or 3 mg. percent in the liver or 0.5 mg. percent in the body as a whole, and this

concentration was maintained at all costs. Copper apparently had no influence on the level of concentration of "fixed iron", it did not appear to be influenced by the presence of an excess of iron, and was not appreciably higher when copper and iron were given together than when milk alone was given.

In 1932 the Journal of the American Medical Association (204) published the following editorial:

The total amount of iron in the body is rather small, probably not exceeding 3 g. or 0.004 percent of the person of adult size. The daily "turnover" or requirement of this element is currently estimated to be not more than 15 mg. or one-fourth grain. Yet under normal conditions of diet and health this quantity of iron suffices to assure the success of a number of extremely important biologic functions. It maintains the integrity of the supply of respiratory pigment - the entire hemoglobin content of the blood; it enters into the composition of every cell. Furthermore, it is widespread in nature and is at least a trace component of most natural foods. In the varied conditions of shortage of hemoglobin that are collectively designated as anemia, regeneration of the missing blood components calls for a supply of iron. Iron therapy has long been practiced in the treatment of anemia. Many observers have been struck by the apparent ineffectiveness of all the familiar types of iron compounds in certain forms of anemia. Thus, in pernicious anemia the administration of iron alone fails to effect a permanent increment of red blood cells. Something more is necessary, as the success of modern liver therapy has shown. The importance of copper as a supplement to iron for the regeneration of hemoglobin in anemic rats was first demonstrated 4 years ago by Hart, Steenbock, Waddell and Elvehjem. These investigations showed definitely, as Elvehjem and Sherman recently pointed out, that, in the presence of copper, soluble inorganic iron salts can be used directly for the formation of hemoglobin. Since that time a number of workers have studied factors affecting the production of hemoglobin in rats rendered anemic by whole milk diets. Most of this work has verified the original conclusions concerning the importance of copper; and today, although there are some who still feel that copper is not the only element in addition to iron which possesses hematopoietic properties, nearly all workers agree that copper is an active agent in hemoglobin synthesis. In most of these studies the activity of copper has been measured by comparing the increase in the hemoglobin content of the blood of anemic rats when pure iron alone is fed with the improvement obtained when the iron is accompanied by traces of copper. Aside from the fact that the hemoglobin molecule does not contain copper, nothing is known about the action of this element. How, then, does the copper function? As Elvehjem and Sherman conclude, copper does not affect the assimilation of iron but does function in the conversion of inorganic iron into hemoglobin.

In recording these studies, which seem destined to throw a new light on many problems of hematopoiesis, it seems desirable to remember that the extent to which the experimental studies are applicable to the human being remains for the most part to be established. Copper is far more widespread in foods than may be commonly supposed. The need for copper is quantitatively far smaller than the requirement of iron. The newer knowledge should not be accepted as a warrant for uncontrolled administration of copper compounds to man. The facts regarding the possible actual needs and

function of this element in human physiology remain to be more definitely ascertained. Hematopoiesis is something more than mobilization of iron and copper. Destruction of blood cells is a process that may be initiated by independent causes. Enthusiasm for the new discoveries must not be permitted to open the door to unfounded therapy.

According to an article by Sheldon (205), the last few years have seen the emergence of two fields of investigation which, though originally independent, are now beginning to merge. In the first place, modern methods of analysis have shown that, in addition to the elements traditionally incorporated in the structure of protoplasm, others, principally metallic, occur with constancy in animal tissues. It is clear that certain elements, particularly copper, manganese, zinc, and rubidium, occur with great consistency in human tissues. This applies especially to copper, which was found in every one of 500 specimens examined by Ramage's method, so that this element, at any rate, may be regarded as a universal and essential constituent of protoplasm. The first reaction to such findings was to regard these elements as contaminants rather than as substances having physiological significance. The second series of investigations referred to above, however, has begun to show that some of them, have functions in the body that render them necessary to life, although they are only necessary in minute quantities. Thus, said Sheldon (205), in 1928 Hart and associates showed that without an adequate amount of copper the body is unable to utilize iron to form hemoglobin; more recently Orent and McCollum and Kemmerer have found that manganese is essential to the processes of reproduction. It was in the light of these researches that Sheldon (205) advanced a few considerations as to how copper and manganese may influence the therapeutic activity of iron, a full review of the subject being beyond the scope of his paper. His considerations are as follows:

The experiments of Hart and his co-workers at Wisconsin showed that carefully purified inorganic iron was ineffective in the cure of the nutritional anemia of young rats caused by the iron deficiency resulting from a whole-milk diet. When accompanied by small amounts of copper (0.025 mg. a day) this pure inorganic iron could be used as the sole source of the iron required for the cure of the anemia, the hemoglobin being rapidly regenerated in the presence of copper. They further found that copper was unique in this property, and could not be replaced by other metals. A few workers such as Titus, Cave, and Hughes, and more recently Myers and Beard, although not denying that copper is the most active supplement to iron, have stated that other elements, including manganese, are also effective. Hart and his colleagues have reaffirmed their original position, which has also been confirmed by other workers. Thus Orten, Underhill, and Lewis, using glass cages, found that copper was the only effective metal, and that manganese, in particular, was quite inert in the formation of hemoglobin. These experiments of Hart and his colleagues are fundamental in demonstrating that the traces of copper so consistently found in animal tissues have at any rate one definite physiological function. It is certain that others remain to be discovered.

An obvious clinical application of their work lies in the nutritional anemia of infants, which has recently been fully described by Helen Mackay. This anemia, like that of the experimental rats, is characterized by a deficiency of hemoglobin due to iron starvation, and it is a natural inference that copper might be of similar value in this condition. Numerous observations have been reported by Parsons, Josephs, Schiff, and others, and although there have been negative

reports, the general trend is to show that in infants the addition of a small amount of copper in the iron preparation used does accelerate the production of hemoglobin. Here, as in so many other cases, it is difficult to transfer the comparatively simple results of controlled experimentation into the more complex field of clinical medicine. Thus it would appear from the works of Parsons on the value of yeast extracts that there is a vitamin as well as a metallic deficiency in this type of anemia, and it has been shown that copper may occur as an impurity in all preparations of iron in therapeutic use, its presence having been demonstrated in about 80 percent of the samples tested. There is, however, sufficient evidence to justify the use of small amounts of copper in addition to the iron needed for the treatment of these babies. Transferring the figures from the experiments on rats to the human being on the basis of weight for weight, the human baby would appear to need about 0.5 mg. copper per diem.

The usual explanation of this action of copper has been that it is a catalytic one. Josephs has recently published some very important work which carries our knowledge a stage further, and which should be borne in mind when copper is employed as a therapeutic adjunct to iron in children. * * * *

The results of Josephs' work are of great interest. It was found that in groups 2 and 3 the retention of iron averaged about 10 percent of the intake, and that copper had no influence on the degree of retention of iron. The effect of copper was to increase the proportion of retained iron found as hemoglobin, so that rats fed on copper plus iron had smaller amounts of iron in the tissues than had rats fed on iron alone. Indeed, these rats fed on iron and copper together had, within the limits of experimental error, the same amount of tissue iron as had those fed on milk alone, indicating that under the influence of copper 100 percent of the retained iron was being used for the formation of hemoglobin. The tissue iron consists of two moieties - the functional iron, which is essential to life and is mostly located in the nucleus in organic combination, and a reserve store of iron which is chiefly found in the liver. In rats fed on milk alone there is from birth a steady drop in the total tissue iron of the body (as the reserve store provided during foetal life is used up), until a steady level is reached, representing the functional iron necessary for the life of the tissues. As the store is depleted, not only by the call for iron for hemoglobin but also for the growing tissues, there is a progressive diminution in the amount available for the formation of hemoglobin, resulting in the development of anemia. When this mechanism is exhausted the level of functional iron in the tissues is maintained at the expense of growth.

It is to be noted that the rats receiving copper plus iron resembled those on a milk diet alone in that there was no reserve store of iron, as all the iron retained from the food was utilized for the formation of hemoglobin. It is therefore probably advisable when giving copper to give larger doses of iron than would otherwise be given, so as to ensure that there is an adequate supply of iron for the two purposes of growth and hemoglobin formation. Mackay's work has shown that there is a correlation in infants between rate of growth and hemoglobin level - the faster the rate of growth the lower the hemoglobin level - owing, of course, to the demands of

the new tissue for nuclear iron. It is a noteworthy fact that young growing tissues appear invariably to have a higher copper content than older tissues (for example, teeth from the first dentition of a child gave distinctly stronger copper lines on spectrographic examination than did adult teeth), and Bodansky, by chemical examination, found foetal brain to be richer in copper than was adult brain.

The results of our general spectrographic survey of human tissues led Ramage and myself to suspect that copper plays an important part in the phenomena of growth, and, if this is the case, the giving of copper to an infant may cause a stimulation of the demand for iron for purposes of growth and hemoglobin formation. If insufficient iron is being given, this may lead to a relative shortage. It is probably largely a question of the relative dosage of the two metals, of which little is known, since it is difficult to transfer the experimental figures in animals to human material. At any rate, it is conceivable that some such explanation as this may underlie the failure of some clinicians to obtain results with copper, and it is at any rate advisable, when giving copper to an infant, to make sure that adequate doses of iron are given in addition. In fact, this work provides a further argument in favor of the modern method of employing iron in larger doses than formerly, as at least 80 percent of the iron preparations used themselves contain copper as an impurity.

The fundamental importance of copper in the animal economy is shown by the fact that at birth the infant is provided with a large reserve of it in the liver. An extensive series of human livers has been investigated spectrographically, from this point of view, in conjunction with Ramage and W. Sheldon. * * * * *

It would appear that copper and iron are the only metals stored as reserve material in the liver during foetal life (it should be mentioned that zinc is very difficult of detection by the flame spectrum used, and no opinion is offered with regard to this metal). The values rise steadily during foetal life and reach their peak at birth, when figures as high as 0.06 percent (dry) for copper and 0.4 percent (dry) for iron may be obtained, which represent more than ten times as much as is found in adult life. After birth these figures drop steadily until the stable value is reached, at the age of 12 to 18 months, which then persists throughout life. A curious feature is that once this level has been reached the amount of individual fluctuation from the average figure is nothing like as much as is found between birth and the attainment of the adult level, due to the fact that though there is in every case a store of copper and iron provided to tide the child over the nursing period, the amount of this initial store is subject to a very wide degree of variation. This is probably an important point, as owing to the small amount of these metals in milk the infant depends on this store to prevent the development of nutritional anemia. It is to be presumed that a child with a poor initial reserve runs a much greater risk of developing anemia, and if the suggestion already made is true - that copper may be an essential factor in growth - the growth of such a child may be retarded. The reserve store is, of course, acquired from the mother, and it is possible that by ensuring that she receives an ample supply of these metals in her diet during pregnancy the incidence of anemia in infants might be lessened. * * * * *

It is impossible as yet to say whether the anemia of adults characterized by a hemoglobin deficiency is connected with a shortage of copper in the body as well as of iron. No postmortem material from which a direct knowledge of the copper content would be obtained has been available, and the estimation of the therapeutic value of copper as a supplement to the large doses of iron employed in its treatment is rendered exceedingly difficult both by the great variations in the extent to which copper occurs as an impurity in the therapeutic preparations of iron, and by the considerable amounts of copper taken in with the food.

Before leaving the subject of copper there is one curious feature that is worth mentioning. As copper is an essential factor in the formation of hemoglobin, one would expect that cases of anemia characterized by deficiency in hemoglobin might show a deficient amount of copper in the blood. (Copper occurs in the blood both in the red cells and in the serum. This has been found by all workers, though there is a disagreement as to which has the greater amount.) Although there has been as yet no work directed to this subject, there are a number of isolated observations in the literature which appear to show that the reverse is actually the case, and that many instances of anemia show a definite increase in the copper content of the blood. Thus Gorter, Grendel, and Wyers found that in the nutritional anemia of children the copper content of the serum average 2.1 mg. per litre as against 1.4 mg. per litre for "convalescents." Warburg and Krebs found in birds that 3 to 4 days after severe bleeding the copper content of the serum might be raised fourfold or fivefold. In a series of 28 bloods, Sheldon and Ramage found that the strongest copper lines of all were given by a case of very severe anemia of pregnancy, while the next strongest were given by a case of severe hemorrhage of puberty in a girl. (The copper lines were also very strong in a case of carcinoma.) In a case of pernicious anemia, on the other hand, the copper lines were only just visible. It is hard to say what these may mean, and in any case they need confirmation on an extended scale. It may be that all the available reserves of copper are being mobilized to cope with the new hemoglobin or that the figure is high because the copper, though mobilized, cannot be utilized by the body owing to the absence of a further factor, such as some vitamin lack. The observation serves at any rate to show how wide are the gaps in our knowledge of the physiological action of copper.

According to Rose and Vahlteich (206), reports are conflicting with regard to the role of various inorganic elements in curing nutritional disorder, but there is no dissent from the view that iron is essential and can be utilized when added to the diet in the form of soluble inorganic salts. The opinion that regeneration can take place with the addition of iron only is denied by most workers from Bunge (1889) to the present time; the results of Hart, Steenbock, Waddell, and Elvehjem, who were the first to show clearly the marked influence of copper in hemoglobin regeneration in nutritional anemia, have been confirmed by every subsequent worker in the field. Studies by Rose and Vahlteich, undertaken to show the value for hemoglobin regeneration of rats made anemic by an exclusive milk diet of wheat in the forms of whole wheat flour, white flour, and prepared wheat bran are quoted. As a result of these experiments, it was found that when whole wheat and oatmeal as supplements to milk were fed to anemic rats in such quantities (1.5, 3, and 6 gm.) as to yield, respectively, 0.048, 0.096, and 0.192 mg. of iron daily, hemoglobin was regenerated in proportion to the amount given, a return to the normal level occurring in 6 weeks

with 6 gm. of either cereal. Prepared bran was fed at levels of 0.9 and 1.8 gm., yielding 0.1 and 0.2 mg. of iron, respectively, regeneration being roughly proportional to the amount of iron. Patent flour fostered hemoglobin regeneration to some extent, better results being obtained with 6 gm. than with 3 gm.

To test the potency of oysters from different localities Coulson, Levine, and Remington (207) fed samples from 14 areas in 13 States to anemic rats. Previous investigations had shown the oyster to be unusually potent in curing the nutritional anemia developed in rats with a milk diet. This effect was due to the inorganic elements of the oyster; elements other than iron, copper, and manganese were not concerned in the regeneration of hemoglobin. In these investigations oysters from only one locality were used, and for this finding to have practical application it was necessary to know what variations exist among oysters from different producing areas and at different seasons of the year, as well as to what extent nutritional effect can be correlated with chemical analysis. In addition to the 14 groups receiving oysters, 3 control groups were also fed by Coulson and his coworkers (207). Two of the control groups were fed iron with the milk. The two iron-fed groups were included to demonstrate that hemoglobin was built in the oyster-fed rats due to the supplementing of iron by another factor. The various oyster samples, arranged in order to descending iron content, show that there is definite correlation between iron content and time of recovery, while no such relationship exists as to copper. In other words, according to these investigators, all oysters contain an abundance of copper, and it is iron rather than copper that fixes the rate of recovery. The oysters from localities north of Maryland were lower in iron and higher in copper than those of Maryland and States south, including the Gulf of Mexico.

Coulson and his coworkers stated (207) that examination of the values for iron and copper of oysters in comparison with 120 different foods and 110 different vegetables and fruits showed that no other commonly used food except liver surpasses oysters in the amounts of iron and copper that they furnish to the diet in an average serving. That these metals are easily available for hemoglobin production was shown in previous work, in which it was found that oysters, oyster ash (acid soluble), and a solution of iron, copper, and manganese in the same quantities, fed to anemic rats, brought about hemoglobin regeneration at the same rate in all three cases.

According to Meyer and Eggert (208) the anemia problem is approached from two different sides - on one side the liver treatment on the other the importance of the combined action of iron and copper. The fact that liver has been used, especially in primary anemias, whereas iron and copper seem to be more effective in secondary anemias, probably explains why so few attempts have been made to connect both methods of research. The investigations indicate that the substance active in pernicious anemia has no relation to the copper and iron content of the liver, whereas the value of liver in secondary anemia may be at least partly due to the presence of these metals.

Bloxson (209) reported that administration of copper in conjunction with iron for hemoglobin stimulation to a series of well infants showing a decrease in hemoglobin produced enough evidence of its value to make it worthwhile to report the results as a means of stimulating further study along this line. A series of 57 infants was fed copper and iron daily in the form of copper sulfate and iron and ammonium citrate. This group of 57 infants showed an average increase of 5 to 15 percent in hemoglobin over a group of 104 infants not receiving copper and iron. Copper is apparently a necessary element in the nutrition of the human body and must be supplied to growing infants in sufficient quantities in conjunction with iron. The evidence presented in this study is in apparent accord with the experimental

evidence in animals that there is a marked increase in hemoglobin when copper is added to a diet containing iron. The marked increase in hemoglobin response of infants given copper and iron apparently indicates that many infants suffer from a partial deficiency of these necessary elements in their early diets and that a substance rich in these hemoglobin-regenerating factors may be of value as a routine addition to the diet after birth.

One hundred and twelve patients with secondary anemia due to various causes were treated by Richter, Meyer, and Legere (210) with a preparation of concentrated whole liver, iron in glycerol, and defibrinated blood. Careful analysis of 1-1/2 ounces of this preparation, the daily average dose administered, showed that it contained the extract of 84.4 gm. of whole liver, a total of 104.24 mg. of metallic iron, and a total of 1.4 mg. of metallic copper. A large percentage of this group of patients showed hematologic improvement on receiving the whole liver-iron preparation and other indicated therapy. The improvement of 11 of the patients could be explained primarily on the basis of having received the whole liver-iron preparation.

According to Schultze (211), comparative investigations on the biological properties of certain elements have shown that their action is more nearly the same the closer the elements stand in the periodic system. For instance, the importance of arsenic, antimony, and bismuth in the therapy of certain infectious diseases, the effect of calcium and strontium on the growth of bone, or the influence of iron, arsenic, and copper on the hematopoietic system have a certain similarity without, however, being equivalent substitutes for each other. Schultze referred to experiments, especially in America, that have shown that not only As and Cu but almost all elements with the atom number between 22 and 34 (Steenbock and coworkers), in combination with iron, exert a strong stimulation on erythropoiesis. These experiments were limited mostly to investigation of the hemoglobin content. It was shown that rats and chickens became anemic when fed small amounts of iron in addition to pure milk but that this alimentary anemia quickly disappeared when to iron a second metal, especially copper, arsenic, or cobalt, was added. Since these substances were not detectable in the blood, their effect was spoken of as "catalytic", especially in connection with copper. According to the experiments of Stoeltzner (quoted by Schultze), copper should be ascribed an important role in connection with the mode of action of liver therapy for pernicious anemia. As only the hemoglobin content was considered in these experiments, it was not evident whether the "catalytic" effect of the metals mentioned was limited to synthesis of hemoglobin only or whether formation of erythrocytes was affected. Schultze (211) wanted to determine whether the functions of hemoglobin synthesis and erythrocyte formation could be separated and whether the elements to which the catalytic effect has been ascribed also exert an influence on the hematopoietical system without the presence of iron somewhat analogous to that of Sr on the growth of bone. He carried out experiments on nickel, cobalt, copper, and iron. Anemia slowly appeared in young animals as long as they were fed 0.5 mg. Ni daily. When the daily dose was raised to 2 mg., there was a quick increase of erythrocytes and a slow increase of hemoglobin, which, however, did not reach the beginning value again. Ni showed no effect on the red-blood picture of adult animals. With a dose of 0.5 to 2 mg. Co daily, young animals died after about 10 days; all the adult animals showed an increase in erythrocytes to above normal value as well as a corresponding increase of hemoglobin with sinking weight curve. The effect of 2 mg. Cu on the formation of erythrocytes was especially striking, while with young animals the hemoglobin was only slightly affected. In only 1 adult animal suffering with moderate anemia did the same dose (2 mg.) have any effect, in which case hemoglobin and erythrocytes showed a similar increase. Although continuous administration of iron (1 mg.) was without effect on adult animals, the alimentary anemia of young rats was quickly cured. These showed at the beginning always a relatively quicker increase of erythrocytes than of hemoglobin so that the color index was temporarily advanced.

In estimating these experiments, Schultze stated that the results obtained for young animals are fundamentally separate from those obtained for adults. With full-grown animals under normal conditions, the blood synthesis and breaking down of the blood approached the average weight. The grown animal had, moreover, enough iron that it was not necessary to add more. On the other hand, young, rapidly growing animals, which have no iron supply, as a result of growth may easily suffer from a relative deficiency of iron. This explains why a pure milk diet leads so quickly to severe anemia in young animals, whereas the older animals can stand it without effect for a long time. Young rats can form erythrocytes, but if no iron is present there is no hemoglobin synthesis. In older animals that have become anemic, the hemoglobin and erythrocytes can return to normal without administration of iron. In erythropoiesis the formation of new erythrocytes and hemoglobin synthesis are sharply separated from one another. The catalytic effect of various metals rests on their stimulating effect on the formation of erythrocytes and on the attempt of the organism to keep its color index constant. Polycythemia cannot be attained by iron alone, whereas continuous application of another metal closely connected with iron leads to abnormally high erythrocyte and hemoglobin values. An increase of hemoglobin, as long as no iron is given, is possible only with adult animals. The catalytic effect, therefore, of the various metals rests on the formative influence they exert on the red bone marrow. The effect of liver therapy in treating anemia is apparently mostly the action of copper.

Heath (212) analyzed 84 patients presenting hypochromic anemia with respect to the hemotopoietic response to oral administration of iron. The percentage of utilization of orally administered iron, as determined by the total amount of iron gained in the circulating hemoglobin, varies inversely with the size of the dosage. The average percentage of iron utilization in the 84 patients during the entire period of recovery was 3.4. The influence that the addition of copper to iron may have in the treatment of hypochromic anemia in adults is at most a minor one, and it is inadvisable to give copper salts as a routine measure in hypochromic anemia in adults.

Bercovitz (9) referred to an article on anemias by Ottenberg in which the latter called attention to the observation that has been made regarding the relationship of copper to the anemias and to the very minute quantities of copper required. Bercovitz then gave his own experience, stating that during 9 years' practice in Korea, the last 3 of which had been entirely in internal medicine in a hospital with an attendance of 50,000 patients yearly in the outpatient department, it was not possible to make the diagnosis of pernicious anemia or sprue in a single instance. He did not wish to create the impression that pernicious anemia did not occur in Korea, but of more than 3,000 patients examined personally and on whom complete blood studies were made, it was not possible to confirm a diagnosis of pernicious anemia.

In this connection Bercovitz (9) mentioned two facts that he considered of at least passing interest. First, the Korean diet, in addition to rice, is made up largely of green leafy vegetables of various sorts and, when the Korean is able to secure meat, the liver, stomach, intestines, lungs, and heart are considered the choice portions. Every effort is made to secure stomach, intestines, and liver for those who are ill, and these organs are usually prepared and eaten whole. Second, virtually all Korean food is served in brass dishes and eaten with brass spoons or chopsticks. The use of brass is a matter of ancient custom, and there is a definite idea among Koreans that the brass dish and spoon have a real value in preserving health. Bercovitz concluded that there is a striking parallel between the recent advances in the study of the anemias and the simple observations of ancient Korean customs.

Brown (213) stated that at least 10 inorganic elements are absolutely essential for life - sodium, potassium, calcium, magnesium, phosphorus, chlorine, sulfur, iodine, iron, and copper. From the standpoint of the practicing physician the supply of only 3 of these elements - calcium, iron, and iodine - had to be watched, because any reasonable diet will furnish the other 7 elements in adequate amounts. In addition to the well-recognized deficiency diseases produced by a lack of vitamins and minerals, it is stated that the importance of these food elements on growth and resistance to infection has also been studied. Each of these vitamins and minerals is essential for life, so it is only reasonable to expect that if any one is omitted from the diet the individual will eventually lose weight and die.

In reply to a request to suggest a suitable combination of iron and copper in solution for use in treating infants and children, noting the dosage of iron salt and of copper recommended for the body weight of the patient, the editor of the Journal of the American Medical Association (214) noted that one could not speak of any exact dose relations of iron and copper per body weight of patient, the usual aim being to give the patient as much as will not derange digestion or produce other unfavorable results. He also observed that the Council on Pharmacy and Chemistry had not accepted as positive the clinical evidence that the use of copper-iron preparations has advantage over that of iron preparations alone.

Experimental proof that minute amounts of copper are necessary to get the full effect of iron, according to Ottenberg (215) has led to the widespread addition of copper sulfate to iron in treating hypochromic anemias. Although there is a good deal of doubt at present whether this is clinically necessary or justifiable, it must be remembered that the amount of copper needed is so small that its effect was discovered only when extraordinary and special precautions were made to remove all traces of copper from the food and from the administered iron; the ordinary preparations of iron and the ordinary diet probably contain all the copper required, and, as copper sulfate is somewhat upsetting to the digestion, he questions whether copper is a necessary addition to the therapeutic armamentarium.

Walker (216) agreed with Ottenberg as to the necessity of using copper with the iron and said that:

There is some difference of opinion as to whether copper enhances the effect of the iron. Mills is of the opinion that it does, and he gave daily $1/16$ grain of copper carbonate (2.1 mg. Cu) in 90 grains of Blaud's mass. One of the patients observed by Dameshek likewise failed to show satisfactory improvement until a daily dose of 0.1 gram of copper sulfate (23 mg. Cu) was added to the iron. The other six cases observed by Dameshek improved satisfactorily on iron alone. Considerable weight must be given to the findings of Mills as his observations were apparently well controlled. Five of his patients were kept for many months on 60 grains of Blaud's mass daily, without improvement. Prompt improvement occurred when copper was added. Witts, however, is emphatic in his belief that copper plays no part in the improvement, for the iron preparation used by him was practically free of copper, containing only 0.01 mg. of Cu in the daily dose. This quantity of copper is less than that in the ordinary diet. The Blaud's pills used by me were likewise prepared from a very pure form of ferrous sulfate, the daily dose of 12 pills containing only 0.1 mg. Cu. The iron preparations used by McCann and Dye contained 0.36 mg. Cu in the daily dose. It appears that improvement in most instances is satisfactory without the addition of copper. Whether the improvement will be accelerated by the addition of copper requires more study before being accepted as a proved fact.

The Council of Pharmacy and Chemistry of the American Medical Association (217) reported that a search of the literature failed to reveal that, in man, the addition of copper to iron has any therapeutic advantage. After a general discussion the council decided that, in view of the lack of specific evidence of the value to man of combinations of copper and iron, it would not for the present accept combinations containing copper and iron. The council discussed the copper-iron patent of the Wisconsin Alumni Research Foundation and felt that issuance of this patent was unfortunate; that the use of the name of a distinguished university whereby there is given or implied scientific approval of a product not demonstrated to be effective is to be deprecated; that the Government should not have issued the patent; and that the criticism of the foundation is that it has patented and collected royalties on a product, which in the opinion of the council, has no originality and no established merit.

Heath (212) had the following to say in regard to the use of copper in hypochromic anemia:

Because of the effectiveness of copper in addition to iron on nutritional anemia in rats and on nutritional anemia in children, there has recently been considerable speculation regarding the possible value of copper in hypochromic anemia in adults. The experimental data at hand suggest that the addition of copper adds no beneficial effect to that of iron in adult hypochromic anemia. In a case of idiopathic hypochromic anemia the addition of copper to iron failed in bringing about a more complete restoration of the hemoglobin to normal than iron alone. Several similar experiments have likewise shown no conclusive evidence that copper added to iron is of value. This is not in accord with the work of Mills and of Adamson and Smith, who believe that iron and copper are more effective in the treatment of idiopathic hypochromic anemia than iron alone.

It is well known that small amounts of copper and other metals are present as impurities in pharmacopeial preparations of iron salts, and that it has been thought that these impurities may have a share in the effectiveness of these preparations. However, a solution of iron and ammonium citrate, freed from the metals of the copper-arsenic group by hydrogen sulfide produced an excellent response in one case of idiopathic hypochromic anemia. It is felt that the influence that the addition of copper to iron may have in the treatment of hypochromic anemia in adults is at the most a minor one. Copper may perhaps hasten the recovery of patients with certain types of anemia. The same final results may be attained, however, by large doses of inorganic iron salts. This fact, together with the possible eventual toxic effect of copper, renders it inadvisable to give copper salts as a routine measure in cases of hypochromic anemia.

Experiments by Underhill, Orten, Mugrage, and Lewis (218) revealed that an exclusive milk-iron-copper diet will maintain rats in an apparently normal condition for prolonged periods. They also found (219) that cobalt when fed with copper to rats on a milk-iron diet produces a true polycythemia characterized by an actual increase in the number of circulating red blood cells.

According to Handovsky (220):

Steenbock and coworkers made the first experimental reference to the significance of copper compounds for the treatment of anemia.

Whipple considered the effect of iron to be a direct replacement of materials; for he assumed that there was only an effect when the iron reserves of the organism of the anemic animal were entirely exhausted. Litnzel also found that the entire iron reserve of the animal must be exhausted in order for iron to accelerate the formation of hemoglobin.

Copper has been repeatedly recommended in human therapy for the treatment of disease. Kobert recommended it very indefinitely where "antiparasitic and tonic effects" are expected and supported his statement by referring to the work of the following: Galippe and Lehmann determined the slight toxicity of copper; Rademacher recommended in 1843 tincture of copper acetate for many different diseases although it should be without effect on the well. Between 1870 and 1890 nothing was heard of copper therapy. Between 1890 and 1900 much copper was again prescribed; it was recommended for diarrhea, various skin diseases, tuberculosis, syphilis, chorea, facial neuralgia, and always again for schlorosis and other anemias. Kobert recommended for the stomach an "organic" copper preparation.

It is especially noteworthy that the regeneration of erythrocytes is independent of that of the hemoglobin, that various copper compounds have a different effect, and that copper and iron must have different points of attack. The feeding of copper compounds or iron compounds or copper and iron compounds causes within 24 hours an increase in the amount of the erythrocytes and the hemoglobin in the blood. These increases are always reversible. Duration and maximum apportionment of the increased content of erythrocytes and hemoglobin are dependent upon the concentrations used. Calculated on the same copper content, the effect of the copper compounds used on the increased formation of erythrocytes or hemoglobin increases in the following order: copper acetate, copper glycol, copper tyrosine, copper protein. Copper acetate in the doses used had scarcely any effect on the increased formation of hemoglobin. The order of effectiveness is the same as with venesection anemia, which speaks much for the usefulness of the method. Iron (for example as soluble ferrum oxydatum saccharatu), especially in essentially larger doses, had an effect on the formation of erythrocytes and hemoglobin in which the action on the hemoglobin was greater. From the experiments it results that copper, especially when it is combined with aromatic amino acids, has a very stimulating effect on the erythrocytic-forming apparatus in anemic animals (venesection anemia, phenylhydrazin anemia, and congenital anemia), and to a less degree on the hemoglobin-forming apparatus. Iron acts the opposite, its stimulating action on the whole is essentially weaker than that of copper. In my experiments with copper protein, 0.25 mg/kg was ineffective, also when given for 6 successive days; 0.35 mg/kg was ineffective for one administration, given on two succeeding days it had the same effect as 0.65 mg/kg in one dose but in any case much less than 0.76 mg/kg. Prompt action always resulted within 24 hours with 0.5 mg/kg. This increased quickly with up to 0.65 mg/kg, from then on more slowly. With the dogs used the optimal specific activity was with 0.65 mg/kg.

From the above experiments with anemic dogs it appears that the action with the administration of larger amounts may last very long. This is easy to understand as copper, like all heavy metals, must be eliminated very slowly. For human therapy it seems that one need not proceed with too small doses. The danger of over-administration does

not exist with the almost generally recognized non-toxicity of copper in the amounts chosen in the above experiments.

Experiments with Cuvitan gave the expected brilliant effect on the regeneration of erythrocytes and hemoglobin; it contains copper protein, which was used in the experiments described above, and iron in the form of a protein absorbate (statement of the manufacturer). Experiments on an anemic dog showed that the two metals potentize each other in the relation Cu:Fe - 1:10 to 1:20.

Many of the animals were sectioned and in no case was there any pathological finding in the dogs fed any copper preparation.

Mackay (221) attempted to demonstrate that iron and copper together produce a better response than iron alone in nutritional anemia by comparing a series of babies treated with both minerals and a series treated with iron alone. The comparison does not suggest that copper was of any benefit in this type of anemia. The types of response obtained with copper were like those customarily seen with iron and ammonium citrate alone, from which it seems fair to conclude that the deficiency affecting these babies was a deficiency of iron and not of iron and copper. The facts set forth when taken together indicate that copper deficiency does not play any important part in the common nutritional anemia of babies. Such negative results, however, do not show that copper deficiency never occurs in babies suffering from iron deficiency. It is possible that a baby might be born with insufficient copper as the result of a maternal dietetic deficiency and so develop a nutritional anemia due to both copper and iron deficiency. However, iron salts with only minute and presumably insignificant traces of copper have been found effectual in treating this anemia, but the indications are that copper deficiency is uncommon, when considering possible copper deficiency, Mackay thinks the varying copper content of milk is of considerable interest. Human milk being richer in copper than cow's milk, it can be assumed that copper deficiency is more likely to occur in bottle-fed babies. This is also true of ordinary cow's milk on the market as compared with the copper content of milk obtained under special precautions to prevent any copper contamination. The observation of Quam and Hellwig in regard to the copper content of milk when pasteurized in contact with copper are quoted. In the dried milk used for the control cases in Mackay's work on infantile anemia, estimations of copper in two samples gave 0.19 and 0.25 mg. of copper per liter of reconstituted milk, compared with a value of 0.12 to 0.18 mg. of copper per liter of fresh milk, as estimated by Elvehjem, Steenbock, and Hart by the same technic; therefore Mackay thinks it is possible that the copper contamination of cow's milk given to babies may actually be a factor of importance in preventing copper deficiency. The fact that large doses of iron salts are desirable in treating anemia has been explained by some authors on the hypothesis that the traces of copper present in these salts are of fundamental importance; but, if Mackay's conclusions are correct, this hypothesis is incorrect.

In the first report of a series on the action of various iron compounds, Fontes and Thivolle (222) stated that the aim of their researches was the cure of secondary anemia as rapidly, completely, and practically as possible, and they found caseinate of iron to be a practical therapeutic method. The results were rapid and stable. However, they tried to get still better results and to this end carried out studies to determine whether copper added to the iron therapy had a favorable effect on the action of the latter. Reference is made to the works of Waddell, Elvehjem, Steenbock, and Hart; McHargue, Healy, and Hill; Keil and Nelson; and Whipple and coworkers; but Fontes and Thivolle found that little attention had been paid to the effect of copper on the therapy of anemia by hemorrhage. They carried out experiments on 2 dogs, each of which received 42.2 mg. of iron and 5 mg. of copper in the form

of caseinate and concluded that there is no doubt but that addition to the iron treatment of small quantities of copper in the form of caseinate clearly improves the hematological recovery of this treatment. Ten mg. per day would be enough copper for an adult man on an unrestricted diet on account of the relative importance of copper in different foods. As during the period of therapy with iron in which copper was not used they received as a maximum 0.5 to 0.6 mg. of the latter metal in the daily ration, Fontes and Thivolle said it was impossible to know what would have been the results without the low involuntary copper content but affirmed that quantities 100 times greater not only caused no inconvenience but accelerated the recovery in hemoglobin and blood globules. It is therefore apparent to them that copper takes a considerable part, which is not catalytic alone, in any process of hematopoiesis. This is certainly true in the manufacture of hemoglobin itself. Experiments carried out by these authors apparently proved their hypothesis that the role of copper is to take the place of iron in the fat and thus liberate an equivalent quantity of iron that becomes utilizable for hematopoiesis. They summarized the results of experiments on the action of tryptophane and histidine alone, associated with caseinate of iron and with caseinate of iron and copper, as follows:

1. The coordinated elaboration of hemoglobin requires the presence of four factors: Iron, copper tryptophane, and histidine. This list does not pretend to exhaust the number of factors in hematopoiesis.

2. The presence of copper, indispensable to the maximum elaboration of hemoglobin, does not appear to be required for the elaboration of the globules, elaboration of which is dominated by iron and especially by tryptophane and histidine.

3. The treatment of secondary anemia by the ingestion of caseinate of iron and caseinate of copper with simultaneous injections of tryptophane and histidine permits a recuperation of the hemoglobin which, for the period of therapy, is 70 percent superior to that with caseinate of iron alone and 32 percent superior to that with caseinate of iron plus caseinate of copper. For the entire period of therapy and the postperiod the results obtained were superior by 82 percent to the caseinate of iron alone and by 8 percent to the caseinate of iron plus the caseinate of copper.

4. Recovery of the tryptophane and histidine from the hemoglobin of a normal dog reached 100 percent. With the anemic dog this recovery varied between 50 and 70 percent, according to the experimental conditions.

From studies then carried out on the action of heavy metals on cysteine and on sulfhydryl groups of proteins, Rosenthal and Voegtlin (223) said that the ability of copper to bring about oxidations in the coagulated egg albumin and of iron and copper to oxidize the fats in dialyzed tissue residues suggests that these metals might stimulate oxidations when added to living cells. They found that the addition of iron or copper salts to dialyzed tissues brings about an oxygen consumption that is largely concerned with the oxidation of fats.

McGhee and Ferguson (224) found that finely divided metallic copper and iron suspended in milk fed to anemic rats caused a rapid rise in hemoglobin and complete recovery, also a rapid return to normal in a number of human cases. The ill effects due to acid hydrolysis did not result when iron and copper salts were used.

According to Stein and Lewis (225), the feeding of evaporated milk to normal rats produced a fall in hemoglobin without the corresponding drop in the erythrocyte count that occurs when raw milk is the sole article of diet. When copper without iron was fed as a supplement to raw milk, results similar to those with evaporated milk were obtained, an indication that the copper in the evaporated milk is responsible for the temporary maintenance of a high erythrocyte count. When given as a supplement to the milk diet of anemic rats, copper ranging in amounts from 0.025 to 0.5 mg. daily showed a definite erythropoietic action without any influence on hemoglobin formation.

Eichholtz and Birch-Hirschfeld (226) found that, of 71 organic and inorganic substances capable of forming compounds with heavy metals, 35 significantly decreased the toxicity of copper toward the isolated frog heart whereas, of 34 substances of this type, only 4 decreased the toxicity of Zn.

In experiments by Perla and Marmorston-Gottesman (227), adult albino rats, carriers of Bartonella muris infection, were fed an adequate diet containing 0.025 mg. Cu per 15 gm. of food. Addition of 1 mg. of Cu with or without 1 mg. of Fe for 2 days before splenectomy and for 1 month thereafter failed to prevent anemia; however, anemia was prevented in 75 percent of the rats when the supplemented diet was fed for 10 days before splenectomy and for the following month. The small amount of Cu in the diet was insufficient for the needs of the rat in the absence of splenic hormone, but an excess of metallic element compensated for the deficiency of hormone.

McHenry (228) considered a diagnosis of Vincent's infection of the tonsil confirmed when the lesions responded within 24 hours to treatment with copper sulfate and sodium perborate. The application of a 10 percent aqueous solution of copper sulfate to all ulcerated surfaces usually relieved symptoms in 2 days. The procedure was repeated every day until the signs of acute inflammation had disappeared, and every second day until the membrane had disappeared completely. The patient was instructed to use half a teaspoonful of sodium perborate in a glass of warm water as a gargle and mouth wash every 2 hours.

The following statement published by Elvehjem (229) in 1933 summarizes the information on the value of copper in anemia:

During the past few years much attention has been given to the iron and copper content of foods and to iron and copper preparations used for blood restoration. It is logical for us to ask, what is the practical significance of these studies? and how much consideration must we give to the supply of these elements in our diet?

Before discussing the justification of the blood regeneration claims accorded iron and copper, it is important to point out that these elements are concerned only with hemoglobin formation. They have little to do with other blood constituents; even the red blood cells are only indirectly affected by these metals.

* * * * *

We must also remember that iron and copper are equally essential for hemoglobin formation although the quantitative requirement of each element may differ to a large extent. As iron is a constituent of the molecule and copper acts only in a catalytic capacity, it is easy to see why more iron than copper is

needed. * * * * * The necessity of copper as a supplement to iron has been demonstrated in so many different laboratories and with so many different animals that there can be no question about the requirement of copper for all red-blooded animals. It is also possible to refer to a number of clinical reports which demonstrate the necessity of copper for humans.

Copper cannot take the place of iron nor can it compensate for unavailable forms of iron. Copper functions only in the production of hemoglobin from the iron after it is assimilated. Absorption and retention of iron and the conversion of iron into hemoglobin are two separate mechanisms. Studies on the availability of iron, therefore, should not be neglected because copper has been associated with hemoglobin synthesis. In order that active hemoglobin formation can take place the body must contain definite quantities of both iron and copper.

* * * * *

During the past year we have studied the effect of supplying additional iron and copper to the diets of a number of the children brought to the clinics. The work is still in progress, but the results to date indicate that the addition of these metals causes a definite increase in the hemoglobin in a very large percentage of the cases. In infants 5 months to 1 year of age the values increase from 10 to 11 gm. up to 12 to 12.5 gm. per 100 c.c. of blood.

* * * * *

What is the best way to insure an adequate supply of iron and copper, which are known to be essential for hemoglobin production? If we are interested only in the simplest and cheapest method, there is no better way than to add simple iron and copper salts to our diet. We now have ample proof that inorganic salts can serve as the sole source of iron and copper. * * * * * Thus the entire iron requirement of one individual can be supplied at the cost of a few pennies per year, and the copper requirement can be satisfied for about one tenth of 1 cent. Can we ask for a simpler method of insuring hemoglobin production?

* * * * *

In choosing foods to supplement those low in iron and copper we have used the total iron and copper content as the basis of our choice. In the case of iron we now know that this is not justifiable. We used to think that all food iron was readily available and utilizable. This is far from true. Food materials contain varying amounts of hematin iron and this form of iron cannot be assimilated. One mg. of iron supplied by wheat is not nearly as valuable as one mg. of iron supplied by a simple salt. The unavailability of a good share of the iron in many of the foods used in infant dietaries probably explains the prevalence of subnormal hemoglobin values observed in the children we have studied. Experimental work which we have conducted with rats during the past year clearly indicates that diets of milk and cereals or cereal products are decidedly deficient in blood-forming elements. These diets are more deficient in iron

than copper, and a fair rate of hemoglobin regeneration was obtained by the addition of iron alone.

The maintenance of an optimum level of hemoglobin in rapidly growing infants is only possible if the demands on the liver iron and copper are reduced to a minimum by the early inclusion in the diet of foods rich in these elements. * * * * *

The availability of the copper in different foods has not been studied. From a practical point of view this question may not be as important as the availability of iron because the requirement for copper is less and many foods which make up the bulk of our diet are relatively rich in copper. In many cases the hemoglobin building power may be restored to normal by the addition of iron alone, but until we are certain of an ample supply of available copper I feel it is wise to supply a small but definite amount of copper along with the iron.

So far I have discussed only the maintenance of a normal blood stream or the restoration of the blood when there is only a slight decrease in hemoglobin. When it becomes necessary to treat anemias with very low hemoglobin values, even foods considered to be rich in iron cannot supply enough to allow rapid regeneration. A number of workers have found iron salts to be superior to liver for the treatment of these anemias. Iron salts are now used extensively for the treatment of hypochromic anemias. The question at present seems to be whether copper should be supplied. In these cases the demand for iron is so great that the requirement for copper may seem insignificant. It is also possible that the diet supplies the needed amount, but unless you can be certain of this fact the copper should be added. * * * * *

In conclusion, we may say that diversified diets supply sufficient iron and copper for the maintenance of a normal blood stream in adults with normal hemoglobin requirements. For rapidly growing children and for adults with increased hemoglobin demands, many diets may be low in iron and possibly copper. The value of foods for supplementing diets low in iron and copper must be based upon the amount of available elements, not upon the total content. Diets deficient in blood-forming elements may be rendered complete by addition of standardized quantities of iron and copper salts.

A booklet on Copper and Health, issued in 1947 by the Copper & Brass Research Association (230), includes a table giving the copper content of the edible portion of about 100 foods. This table should be helpful in selecting food when a copper-rich diet is desired.

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